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NOTES ON THE GENUS *RHIPICEPHALUS*, WITH THE DESCRIPTION OF NEW SPECIES, AND THE CONSIDERATION OF SOME SPECIES HITHERTO DESCRIBED.

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(With 12 Text-figures.)

THE identification of species of *Rhipicephalus* is likely to give more trouble than is the case with any other genus of Ixodidae, for while, on the one hand, there are few species which depart greatly from the general type, on the other hand the range of variation within the species is extremely great. In no genus is it so dangerous to describe a new species from a single individual, especially if the specimen be a female.

The structural features which are fairly constant in a species are few, and not very easy of determination; for example, the exact shape of the *basis capituli* in the male is of the first importance, but a slight error of orientation under the microscope will considerably alter its apparent outline. There are two reasons for this: first, the dorsal surface of the body and that of the capitulum are usually in slightly different planes, so that when the body is horizontal the capitulum is depressed and fore-shortened; in the second place, as Dönitz (1910, p. 465) has already pointed out, the antero-lateral border of the *basis capituli* is not, like the postero-lateral border, a definite edge, but is a rounded surface, and a faulty impression of the degree of salience and

of the precise position of the lateral angle is sure to be obtained unless the capitulum is placed in an accurately horizontal position for examination.

Certain structures which are of great specific importance in other genera are practically identical in all species of *Rhipicephalus*. The dentition of the hypostome is always 3 | 3, and the coxal armature is so uniform that but slight assistance is to be expected from the study of it. A useful point, however, is the absence or presence of an anterior projection on coxa I, visible dorsally. This readily strikes the eye, is subject to comparatively little variation within the species, and at once relegates the specimen, at all events if a male, to a particular group of species.

With very few exceptions the genus is inornate, so that a specific character of great utility in *Amblyomma*, *Aponomma* and *Dermacentor* is here practically lacking. The yellow legs of *R. evertsi* Neumann, 1897, are noticeable, and some species have, as a rule, exceptionally dark scuta, but coloration on the whole—especially in specimens preserved in spirit—is a doubtfully useful specific character.

R. oculatus Neumann, 1901 and *R. evertsi* Neumann, 1897 are clearly separated from all other known species of *Rhipicephalus* by their hemispherical bead-like eyes. In a few other species the eyes are slightly prominent, but usually they are almost flat. Their comparative size is of some importance, and, to a less extent, their colour.

The size and shape of the spiracle, though by no means invariable, will often be found useful in diagnosing a species; the shape differs with the sex, that of the male always being the more elongate and comma-shaped. In some species the spiracle of the male narrows but slightly towards its termination, while in others (*e.g. R. sanguineus* Latreille, 1804) the tail of the comma is well-marked. There is usually present a more or less marked infolding of the spiracle rim on its dorsal border, but this "rim-fold" as we may call it is too variable to be of great assistance. It is often stated in the original descriptions of species of *Rhipicephalus* that "the scutum of the male covers the whole dorsum," or that this is not the case, the body extending beyond the boundary of the scutum; and in the same way the presence or absence of a caudal appendage is frequently given as a specific characteristic. As a rule it is merely a question of an unfed or of a distended male, and though distended examples certainly appear to occur more commonly in some species than in others, and caudal appendages when present to be more pronounced, it would be exceedingly unsafe to say

that these characteristics are absent in any species unless a large number of males had been examined.

Mere size seems to be of less account in *Rhipicephalus* than in any other genus of the Ixodidae. In the accompanying figures outlines are given, drawn to scale, of large and small males of three species, the individuals compared being in each case taken from the same tube of ticks, collected on the same occasion from a single animal, and connected by every grade of intermediate size. The larger specimens usually have the specific characteristics (anal plates, punctations etc.) more strongly developed than the smaller, and in most species—if not in all—well-developed individuals may be found with the body extending beyond the scutum and more or less prominent caudally.

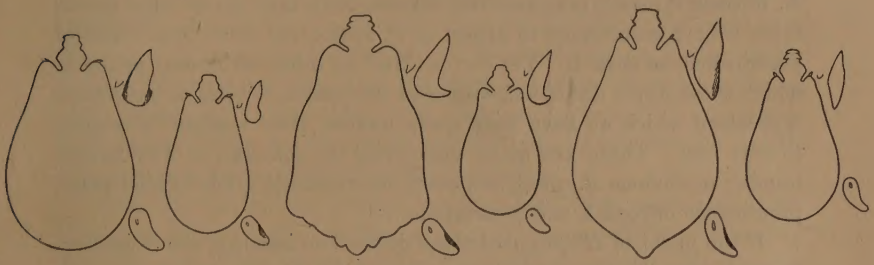

Fig. 1. Outlines of large and small ♂'s of three spp. of *Rhipicephalus* (from left to right *R. simus* Koch, 1844, *R. haemaphysaloides* (Supino, 1897) and *R. appendiculatus* Neumann, 1901). Each pair is drawn to scale from specimens taken at the same time from a single host.

It is most unfortunate that the anal plates, which, as highly chitinised structures, might be expected to be of great taxonomic importance, are subject to very considerable variation, though there is generally recognisable a normal form of anal plate for any given species. *R. lunulatus* Neumann, 1907 has such very striking anal plates that no one could hesitate, on coming across a single well-marked individual, to describe it as a new species. Yet it differs in no other respect from *R. simus*, and we possess specimens taken from a single animal, clearly connecting the two forms of anal plate. *R. falcatus* Neumann, 1908 presents a similar phenomenon. Indeed the extreme variability of *R. simus* has led to the establishment of several species, some of which have already been suppressed, while others will at least have to be degraded into varieties.

As regards the males, we have still to consider the dorsal sculpture. The cervical and lateral grooves are of importance, and there are usually present on the posterior portion of the scutum three furrows or pits which are fairly constant for the same species and which may be called the "dorsal furrows."

The punctation of the scutum presents a great difficulty. There is certainly for each species a characteristic punctation very recognisable in typical examples, but often widely departed from in individuals, or in local varieties, and when this is the case the difference of facies between two ticks otherwise structurally identical may be very great. A striking case is the tick named by Neumann *R. falcatus*, a densely punctate form which at the first glance bears no resemblance at all to *R. simus*, where the punctations are few, and arranged in linear series. Moreover *R. falcatus* typically possesses very characteristic anal plates quite unlike those we are accustomed to expect in *R. simus*, and there is no anterior prominence on coxa I. Yet we can find no other structural points in which these forms differ, and moreover we possess a tube of ticks from Nyasaland which we have been quite unable, after repeated attempts, to sort out. There are many undoubted *R. falcatus*, a considerable number of obvious *R. simus*, and every intermediate grade of anal plate, prominence of coxa I, and punctation.

If the males of *Rhipicephalus* are difficult to identify, the characters presented by the females are even more unsatisfactory, for not only are they without anal plates, but the shape of the *basis capituli* differs little in the various species, and the anterior prominence of coxa I is never noticeable. The shape of the scutum should be noted, the presence or absence of a lateral groove, and the scutal punctation. Further assistance will be received from a study of the porose areas and of the spiracles, but, as we have already said, a single female, unless it presents some unmistakeable peculiarity, is a very unsatisfactory basis for the formation of a new species.

In identifying a male *Rhipicephalus* the best guides will be found to be, the anterior prominence on coxa I, the exact shape of the *basis capituli* carefully orientated, and the position and nature of the lateral angles (whether obtuse, acute, or about a right angle); the dorsal furrows; the anal plates, the grooves and punctations of the scutum, and the spiracles.

If the belief in the great specific variability of *Rhipicephalus* depended entirely on the study of ticks captured under natural conditions it might be argued that forms in reality distinct had been

confused, and that the species were in fact numerous, though difficult of separation. But it is strongly supported by the results obtained by the rearing of ticks in the laboratory, and this has been done over and over again in the case of common species such as *R. appendiculatus* and *R. capensis*. The wide divergence of individuals raised from a single batch of eggs is most striking, especially as regards the males. The disparity in size of captured male ticks apparently belonging to the same species was often so great as to suggest that the males lived longer than was supposed and grew after reaching maturity; especially as the larger specimens were almost always more highly chitinised and more strongly characterised, but similar differences are observed in newly emerged males which have been reared in the laboratory from nymphs taken from one host.

The genus *Rhipicephalus* is essentially African. *R. sanguineus* is practically cosmopolitan—a fact no doubt attributable to its usual host, the dog. *R. bursa* has overflowed into southern Europe, being chiefly distributed along the shores of the Mediterranean. *R. texanus* Banks, 1908 is certainly no more than a N. American variety of *R. sanguineus*, if it deserves even varietal rank, and the only known distinct Asiatic *Rhipicephalus* seems to be *R. haemaphysaloides*.

Now the writer has, during the last few years, examined many thousands of ticks collected from all parts of Africa, chiefly in connection with the work of the Entomological Research Committee. He has also, thanks to the great courtesy of various collectors and of the authorities of the chief continental museums, been able to study the actual types of nearly every so-called species of *Rhipicephalus*, and his conclusions, as far as he has been able to arrive at any, will, it is hoped, be of some interest to those who have to deal with this most puzzling group.

The first conclusion is that the genus *Rhipicephalus* is in an extremely fluid condition. There are what appear to be a considerable number of species *in the making*—forms distinct enough when characteristic examples are selected, but in many cases merging into each other by imperceptible gradations.

A certain number of forms—about sixteen—have been repeatedly met with in considerable numbers, and though they often include ill-characterised individuals, they each centre round a recognisable type distinct in each case. In the second place there are certain forms (*e.g. R. armatus* Pocock, 1900, *R. cuspidatus* Neumann, 1906, *R. deltoideus* Neumann, 1910) of which few examples have ever been found, but which are so peculiar that their claim to specific rank cannot be denied. Lastly there

are not a few forms which have been described either from very scanty material, or from a considerable number of examples taken on a single occasion, and presenting no very salient characteristics. Our experience in the case of the better known "species" makes it probable that, if a large number of examples were available for study, even those characteristics which appear to distinguish them would fail in the all-important quality of constancy. It is clear, then, that the taxonomy of *Rhipicephalus* is bound to be unsatisfactory, and the question to be solved is what way of tackling it is likely to be of most use to those who have to deal with the group. Forms merging into one another by imperceptible gradations are not, scientifically, distinct species—nor even distinct varieties; yet to insist on this, and to fuse together such obviously different forms as, for example, *R. simus* and *R. falcatus*, would lead to inextricable confusion, and it seems better to assign the term species, under protest, so to speak, to forms sufficiently distinct where characteristic individuals are considered, though cases are sure to arise in which an example can be attributed with equal justice to either of two such "species," and it may be even necessary to describe it by connecting with a hyphen two "specific" names—as *R. simus-falcatus*. The systematist has no need to apologise for a want of definiteness the responsibility for which lies with Nature herself.

It is from this point of view that the subjoined new "species" of *Rhipicephalus* are described, and the case of the first—*R. neavei* (see p. 7)—may be dealt with a little more fully.

Among a large number of tubes of ticks received from Nyasaland, N. Rhodesia and British E. Africa the constant recurrence of a certain form—very characteristic in well-marked examples—was noted. It seemed incredible that a tick evidently so common in those regions had remained undescribed, and yet it seemed impossible to recognise it as at all a normal form of any of the species whose establishment has been based on a considerable number of specimens. It bears a superficial resemblance to *R. appendiculatus*, but differs from it in what must be regarded as among the most constant characteristics of the male—the shape of the *basis capituli*, and the anal plates. Of the species based upon few examples and possessing no very salient characteristics it seemed, from descriptions, to have affinities with one or two—notably *R. kochi*, but the types of this species have been examined with a negative result. We have here, then, a form of *Rhipicephalus* which has at least as good a title to rank as a distinct species as the majority of those already recognised, though if only two or three examples had

ever been found, and these had chanced to be among its more ill-characterised specimens, a very inadequate idea would have been obtained of what may be considered its normal appearance.

A further word with regard to specific descriptions of *Rhipicephalus*. In view of the uniformity of certain structures throughout the genus, and of the great variability of others within the species, it seems desirable to depart from the method—which has been found convenient in the case of other genera—of proceeding at once to describe all the external features in sequence. It will be more useful to preface such a description—which will often be of a somewhat indefinite and general nature—with a brief statement of the salient characteristics upon which the species is chiefly based. A similar method has already been adopted by Dönitz (1910), who alone of previous writers has at all appreciated the very unstable nature, in species of *Rhipicephalus*, of structural features which appear to be remarkably constant in other genera.

Rhipicephalus neavei n. sp.

Figs. 2, 3.

Male. Salient features: *Inornate; Basis capituli much broader than long; lateral angles near the middle, and somewhat acute. Coxa I strongly prominent anteriorly. Punctations numerous, small, rather unequal, absent immediately in front of the eyes. Lateral grooves well-marked, including no festoons. Dorsal furrows distinct, the laterals small, oval and detached. Anal plates of characteristic shape* (see Fig. 2), *the external and posterior borders almost at right angles; the internal border projecting inwards distally. Spiracle narrow, only slightly curved.*

Detailed description:—

Dorsal aspect: *Palps* short, flat dorsally, articles 2 and 3 about equal; posterior border of article 2 straight; article 1 only slightly visible. *Basis capituli* with straight posterior border and strong cornua; lateral angles rather acute and directed somewhat backwards (especially on under surface), the antero-lateral border somewhat convex, the postero-lateral concave; the median field generally punctate, especially posteriorly. *Scutum* measures 3·6 × 2 mm. in a fairly well-developed specimen, punctate all over except the region immediately in front of the eyes and the fold external to the lateral grooves. The strong punctations which in the ♂ generally correspond to the lateral grooves of the ♀ are absent.

Cervical grooves deep oval pits, generally followed by shallow divergent depressions. Eyes somewhat salient, emphasized by a rather deep impression at their dorsal limit. Festoons very short, their intervals rather broad. Median dorsal furrow rather long and pointed anteriorly; lateral furrows small, oval, near the festoons but generally detached.

In well-fed specimens the body extends beyond the scutum and there is a caudal appendage terminated by a "plaque."

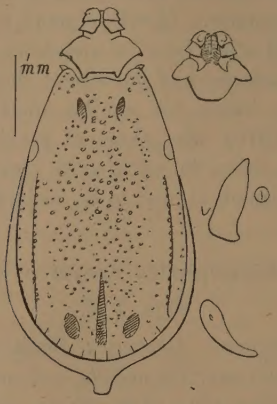

Fig. 2. *R. neavei* n. sp. ♂. Dorsal aspect, ventral view of capitulum, anal plates and spiracle. Original, C. W.

VENTRAL ASPECT: *Integument* often much lighter coloured than the scutum, legs and plates. *Auricular ridges* of basis capituli well-marked, and directed somewhat backwards. *Coxal armature* normal. *Anal plates* typically as described above, but subject to considerable variation, the angle formed by the external and posterior borders being sometimes more obtuse, and the inner protuberance less noticeable; accessory plates only indicated by a very slight chitinous point. *Legs* long.

Female. *Capitulum*: basis capituli like that of the ♂, with the antero-lateral border rather more convex; porose areas small, circular, two diameters apart; palps somewhat larger than in the ♂, article 1 more visible. *Scutum* about 1·3 × 1·1 mm.; eyes rather large and salient, and situated somewhat posteriorly; cervical grooves well-marked, converging at first, then sharply diverging; lateral grooves absent, but there is a convex smooth region on either side; the median field is closely and fairly uniformly punctate and there are a few

punctations on the scapulae, but the area immediately in front of the eyes is glossy and devoid of punctations as in the ♂. In exceptional specimens the lateral punctations of the median field are so emphasized as almost to amount to a lateral groove. *Spiracle* short-oval, with only a slight dorsal projection.

This species bears a superficial resemblance to *R. appendiculatus*, but the shape of the basis capituli and of the anal plates in the ♂ and the absence of lateral grooves in the ♀ are sufficient to differentiate it from that form. It seems to be more nearly allied to *R. kochi* and *R. cuneatus*.

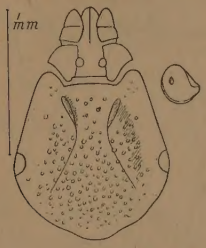

Fig. 3. *R. neavei* n. sp. ♀. Dorsal view of capitulum and scutum, and spiracle. Original, C. W.

Described from a large number of specimens taken in N. E. Rhodesia from the "bush-pig," eland, "bush-buck," "impala," kudu and man; in Nyasaland from the roan antelope, "bush-buck," "wart-hog," *Lepus* spp., cattle and grass; in British East Africa from the goat and the buffalo. The specimens which appeared most characteristic and were selected as types were taken by Mr S. A. Neave from an eland near the mouth of the Tasangazi R., Luangwe Valley, N. E. Rhodesia [E. R. C. No. 168]. Types at British Museum and Cambridge.

Specimens of this tick were sent to Geheimrath W. Dönitz who agrees that it is a form unknown to him. The presence of a lateral groove in the ♂ and its absence in the ♀ caused him to suspect that they belonged to different species, but not only do they constantly occur together, but the strong line of punctations which are the true representatives in male ticks of the female lateral groove is here absent.

R. neavei var. **punctatus** n. var.

Figs. 4, 5.

Male. Like *R. neavei*, but: *Body* narrower and more elongate (scutum 3 × 1·6 mm. in fairly well-developed specimens). *Basis capituli* with lateral angles less acute and not recurved. *Scutum* more deeply and

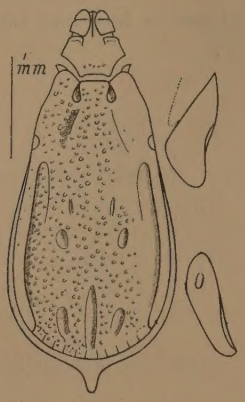

Fig. 4. *R. neavei* var. *punctatus* n. var. ♂. Dorsal view, anal plates and spiracle. Original, C. W.

uniformly punctate, only the lateral margins behind the eyes being nearly free from punctations. *Anal plates* with angle formed by external and posterior borders obtusely rounded; no accessory plates visible in any of the examples seen.

Female. Like *R. neavei*, but: Scutum longer and more oval, punctate all over, including the lateral border. Fairly distinct lateral grooves, or at all events a clearly marked lateral ridge. Porose areas larger and nearer together.

Described from 13 ♂s and 8 ♀s from Kudu, near Fort Mlangeni, Central Angoniland, Nyasaland (Neave, v. 1910, E. R. C. No. 132), 1 ♀ from *Impala aepiciros melampur* (sic) on N.-W. shore of L. Nyasa (Neave, VII. 1910, E. R. C. No. 127), and 1 ♀ from reed-buck, Valley of Rukuru R., N. Nyasaland (Neave, 26. VI. 1910, E. R. C. No. 158).

Some of the specimens of *R. neavei* taken by Old from roan antelope near Marisba, Nyasaland, I. 1911 (E. R. C. Nos. 226–227 *a*) showed a

tendency to approach this variety[1]. Types at British Museum and Cambridge.

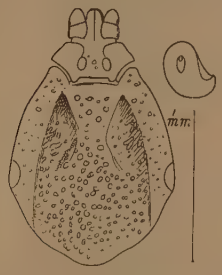

Fig. 5. *R. neavei* var. *punctatus* n. var. ♀. Capitulum, scutum and spiracle. Original, C. W.

R. longiceps n. sp.

Figs. 6, 7.

Male. Salient features: *Inornate; Basis capituli not much broader than long; lateral angles distinctly anterior and slightly obtuse. Coxa I strongly prominent anteriorly. Punctations very numerous, deep, uniform, discrete, on every portion of the scutum. Lateral grooves well-marked, including one festoon. Dorsal furrows deep, linear, nearly parallel, sub-equal. Anal plates* (see Fig. 6) *like those of R. capensis; accessory plates very characteristic, long and superficial. Spiracle narrowing abruptly to a long uniform tail.*

Detailed description:—

DORSAL ASPECT: *Palps* rather long, flat or slightly concave dorsally, article 3 longer than 2, and with posterior raised ridge; article 1 fairly visible. *Capitulum*: Basis capituli of the *R. appendiculatus* type, the postero-lateral border about twice as long as the antero-lateral; posterior border straight, with fairly marked sharp cornua, numerous punctations. *Scutum* (about 3 × 1·8 mm. in average specimens) red-brown, uniformly and deeply punctate all over, including the lateral borders and festoons; cervical grooves nearly circular pits, not continued as posterior depressions; festoons longer than broad and very punctate. Dorsal furrows linear, sub-equal, nearly parallel. *Body*, with light

[1] Some ticks which Dönitz has alluded to as *R. pravus* (Dönitz, 1910, p. 479) but has never formally described, and of which he has kindly sent us specimens, seem to belong to this variety, though their eyes are exceptionally prominent.

yellow integument, extends far beyond the scutum posteriorly in distended specimens, the caudal appendage being unusually strong, but without a terminal plaque. Red-brown plaques correspond to the festoons on either side. Only one of the 37 ♂s was without the caudal appendage, and in this the accessory plates were hardly visible.

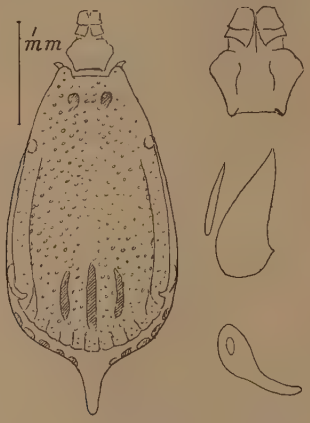

Fig. 6. *R. longiceps* n. sp. ♂. Dorsal aspect, capitulum, anal plates and spiracle. Original, C. W.

VENTRAL ASPECT: *Integument* yellowish-white in all the specimens. *Auricular ridges* slight. *Coxa* I rather short; coxa II triangular; the internal spur almost absent on coxae II and III; the spurs on coxa IV small and well separated. *Anal plates* somewhat clavate, usually with an internally directed point (as in *R. capensis*); they tend to become broader distally in large specimens. Accessory plates long superficial strips of hard chitin, salient posteriorly. *Legs* rather long; pads long. *Spiracles* enlarged anteriorly, then constricted to a long slightly curved tail of uniform width.

Female. *Capitulum* remarkably long (0·8 mm.), due chiefly to the unusual length of articles 2 and 3 of the palps; basis capituli punctate, with straight posterior border and slight cornua; porose areas large, the interval rather greater than the diameter. Palps with article 1 long, but partly concealed by article 2 which is very long and produced backward to a point; article 3 long and narrowing distally. *Scutum* sub-circular, deeply emarginate, deeply punctate all over:

lateral grooves fairly well-marked for two-thirds the length; cervical grooves fairly deep and only slightly convergent. *Dorsum* with numerous very large punctations. *Spiracle* short comma-shaped, rather sharply recurved.

Fig. 7. *R. longiceps* n. sp. ♀. Capitulum, scutum and spiracle. Original, C. W.

Described from 18 ♂s and 3 ♀s (No. 351) from "Klipspringer Bok" taken by Dr F. C. Wellman in 1907 in the Benguella Hinterland, Angola, long. E. 15° 05′ lat. 12° 44′, altitude 1360 metres, and 19 ♂s and 2 ♀s (No. 393) in a mixed collection of ticks taken by the same collector in the same district during 1908 but with no host recorded. Types in Cambridge.

R. sculptus n. sp.

Figs. 8, 9.

Male. Very large, a well-developed specimen measuring 4 mm.

Salient features: *Inornate; Basis capituli not much broader than long. Lateral angles anterior. Coxae somewhat prominent. Lateral grooves, dorsal furrows, anal plates and spiracles much as in R. supertritus. Sculpture of scutum very characteristic, glossy raised ridges defining a very distinct pseudo-scutum and outlining the dorsal furrows; the rest of the surface consisting of extremely rough shagreened tracts from which arise raised areas which are deeply punctate.*

Detailed description:—

In most respects much like *R. supertritus* Neumann, 1907, but: Average size somewhat larger. *Basis capituli* rather broader in comparison. *Coxa* I less prominent anteriorly, the projection curving sharply

outward. *Legs* yellower, contrasting strongly with the dark brown of the highly chitinised portions of the body. *Accessory plates* absent.

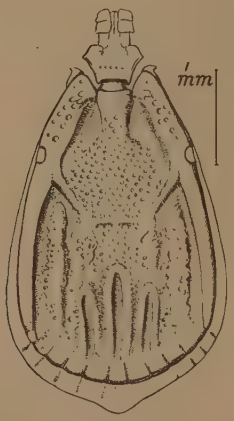

Fig. 8. *R. sculptus* n. sp. ♂. Dorsal aspect. Original, C. W.

Female. Like *R. supertritus*, but: Larger, the scutum measuring 1·8 × 1·8 mm. *Scutum* with lateral ridges less divergent and longer, converging behind the eyes, so that the whole strongly punctate central area is framed by a glossy raised border; a raised punctate area or island

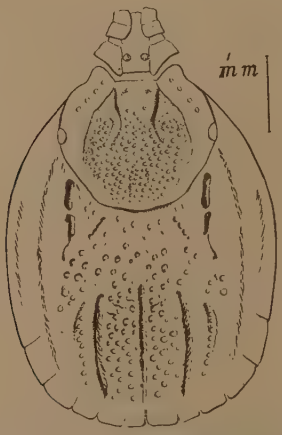

Fig. 9. *R. sculptus* n. sp. ♀. Dorsal aspect. Original, C. W.

is present in the region between the cervical grooves and the lateral ridges. *Dorsum* strongly punctate and grooved (see Fig. 9).

The short white hairs on the dorsum, especially along the marginal grooves, are extremely stout and thickly set.

Described from 11 ♂s and 5 ♀s (E.R.C. No. 230 *a*) from roan antelope, Mpalali R., Marimba, Nyasaland (Old, I. 1911) in company with *R. supertritus*, *H. aegyptium* and *B. australis*. 1 ♂ (E.R.C. No. 227 *a*) from the same locality and host (Old, I. 1911) in company with numerous other species. 3 ♂s and 1 ♀ (No. 115 *b*) from zebra, S. Rukura Valley, N. Nyasaland (Neave, VI. 1910) in company with *R. simus*, *R. capensis*, *R. sanguineus* and ticks of other genera. Types at British Museum and Cambridge.

R. appendiculatus, *R. supertritus* and *R. sculptus* are three forms closely allied and in certain structural points practically identical, but presenting quite a different facies on account of their progressively complicated scutal sculpture in both sexes.

Notes on some obscure species of Rhipicephalus.

R. ecinctus Neumann, 1901 and **R. maculatus** Neumann, 1901.

In his *Révision de la Famille des Ixodidés*, Part IV, 1901, Neumann described *R. maculatus* ♂ and ♀ and *R. ecinctus* ♂. *R. maculatus*, curiously enough, was taken from a beetle, *Platymeris horrida*, in the Cameroons; *R. ecinctus* ♂ was described from specimens from an unknown source in the Berlin Museum. Later ("Notes sur les Ixodidés," VI. *Arch. de Parasitol.* 1908), Neumann recorded the occurrence of *R. ecinctus* on the buffalo at Mt. Njiro, British E. Africa, and described what he took to be its female. In the same tube were specimens of *R. pulchellus*, *R. simus* and *R. oculatus*.

Through the great courtesy of the authorities of the Berlin Museum I have been able to examine the types of these species, and I have also recently received, through the Entomological Research Committee, further specimens which throw an unexpected light on the subject. The specimens in question are these:

(1) 5 typical *R. ecinctus* ♂s and 4 ♀ ticks unmistakeably belonging to them, taken (in company with *R. supertritus* and *R. evertsi*) from a buffalo in British E. Africa by Dr H. S. Stannus, I. 1911 (E.R.C. No. 193).

(2) 9 ♂ ticks showing various grades of maculation between *R. ecinctus* and *R. maculatus*, and taken from the same host during the same month by Dr Stannus (E. R. C. No. 194).

(3) 4 ♂s and 6 ♀s, the ♂s completely uniting *R. ecinctus* and *R. maculatus* and the ♀s as in tube (1), collected from grass at Masongalini, British E. Africa by S. A. Neave, IV. 1911 (E. R. C. No. 263).

(4) 12 ♂s and 16 ♀s, the ♂s mostly *R. maculatus* but some approaching *R. ecinctus*, and the ♀s as before, also taken from grass by S. A. Neave at Mtito Andei, III. 1911 (E. R. C. No. 264 *c*).

From the consideration of these specimens and of the types, two things are abundantly clear; first that *R. maculatus* and *R. ecinctus* are identical, and secondly that the ♀ is as yet undescribed, wrong ♀s having been attributed to both the supposed species.

Now in examining the ♂ types of *R. maculatus* and *R. ecinctus* it was impossible to find any difference except in the maculations of the scutum, and even in the type *R. ecinctus* ♂ the central white spot was distinctly visible. The most characteristic white blotches on a typical *R. maculatus* are a central spot, two rather linear splashes behind the pseudo-scutum, two lateral spots rather behind the middle, and two others more posterior.

In all our specimens the central spot persists, the post-pseudoscutal splashes being next in order of persistency. The other spots are feebly present in some specimens and vividly in others.

R. maculatus and *R. ecinctus* are therefore identical, and the ♀, hitherto undescribed, is diagnosed below.

What, then, are the ♀s which have been attributed to these supposedly different forms?

Now the alleged ♀ of *R. maculatus* is undoubtedly *R. pulchellus* Gerstäcker, 1873 ♀. In the original description it was described as like *R. pulchellus* with certain differences—mostly trifling, and the differences come well within the range of variation we have observed in the numerous specimens of undoubted *R. pulchellus* ♀s we have seen.

The alleged *R. ecinctus* ♀ is a somewhat aberrant *R. simus* ♀. In the original description of *R. ecinctus* ♀ Neumann noted its similarity to *R. simus*. The capitula are precisely alike, the scutum only being rather unusual, but in view of the remarkable tendency to variation already alluded to in *R. simus* there can be little doubt as to the correctness of this conclusion, especially as *R. simus* was present in the tube from which *R. ecinctus* ♀ was described.

To recapitulate, *R. maculatus* takes priority, *R. ecinctus* falling into synonymy. *R. maculatus* ♀ Neumann, 1901 becomes a synonym of *R. pulchellus* ♀, and *R. ecinctus* ♀ Neumann, 1908 is a synonym of *R. simus* ♀. The true ♀ of *R. maculatus* is here described for the first time, and the ♂ is re-described.

R. maculatus Neumann, 1901.

Figs. 10, 11.

Male. Salient features: *Ornate; Basis capituli nearly as long as broad, with sides sub-parallel, rounded in front—coxa I only slightly prominent—punctations few, medium, scattered—lateral grooves absent—dorsal furrows faint or absent—anal plates long, rounded; no accessory plates.*

Detailed description:—

Dorsal aspect. *Capitulum*: palps medium, flattened, article 1 fairly visible, article 2 with postero-internal angle somewhat produced; basis capituli like that of *R. pulchellus*, the sides sub-parallel, only slightly salient laterally very near the anterior end; cornua very slight.

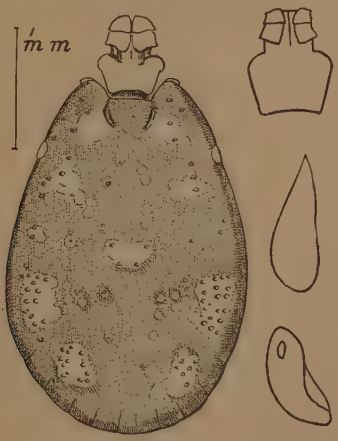

Fig. 10. *R. maculatus* ♂. Dorsal aspect, capitulum, anal plate and spiracle. Original, C. W.

Scutum (3·5 × 2·4 mm. in an average specimen) rather convex, smooth, decorated by white maculations which are more or less obsolete in many specimens; the chief (see Fig. 10) are a median spot and four pairs of

lateral irregular blotches; when all are absent but the median spot the form is that which was described as *R. ecinctus*; cervical grooves deep pits; lateral grooves absent, but indicated by irregular groups of punctations. Eyes medium, rather salient; festoons of moderate length, the externals progressively shorter.

No specimen exhibited a well-marked caudal process.

VENTRAL ASPECT. *Integument* of the same colour as the scutum and coxae. *No auricular ridge* on the basis capituli. *Coxa* I very short; coxae II–IV with spurs rather strong, especially the externals. *Anal plates* with internal border nearly straight, external and posterior borders convex. No accessory plates. *Spiracles* rather narrow and not much curved. *Legs* strong.

Fig. 11. *R. maculatus* ♀. Dorsal aspect. Original, C. W.

Female. Ornate, the *scutum* presenting a yellowish-white median posterior area, shading off to brown in the front, and variable in size and intensity. *Capitulum*: palps rather long, article 2 somewhat prolonged at its postero-internal border; basis capituli only slightly salient laterally; porose areas medium, far apart. *Scutum* sub-circular, smooth, with few punctations; cervical grooves well-marked; lateral grooves absent or faintly visible at their origin, but indicated by irregular large punctations, external to which the scutum is dark brown; eyes medium. *Dorsum* like that of *R. pulchellus*, having similar patches of clavate white hairs postero-laterally. *Spiracle* sub-triangular, the white area somewhat sharply curved dorsally.

The ♀ greatly resembles that of *R. pulchellus* in general structure but may be immediately distinguished from it by the scutum. The

yellowish-white area is much more restricted, being confined to the region between the usual position of the lateral lines, and it quite lacks the hard enamelled appearance presented by *R. pulchellus*, in which almost the whole of the scutum is of a vivid white. Moreover the scutum of *R. maculatus* ♀ is comparatively much shorter and nearly circular. Types (of ♀) at British Museum and Cambridge. The type ♂ is in the Berlin Museum.

R. simus, R. lunulatus and R. glyphis.

Geheimrath Dönitz very kindly sent us the type specimen of *R. glyphis* ♂ for examination. It is a dry specimen, mounted on a long entomological pin, and therefore not easy to examine from all aspects, but after the closest study I could find no difference between it and

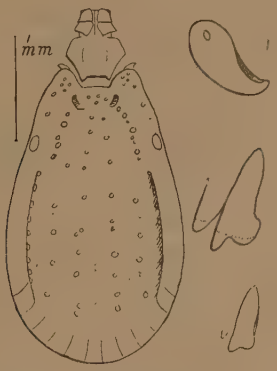

Fig. 12. *R. simus* var. *lunulatus*. Dorsal aspect, spiracle, anal plates (typical), anal plates of another specimen. Original, C. W.

the types of *R. lunulatus* Neumann in the British Museum. I therefore regard *R. glyphis* Dönitz, 1910, as a synonym of *R. lunulatus*. But, in the light of numerous fresh specimens in the collections of the Entomological Research Committee I am unable to consider *R. lunulatus* as anything more than a variety of *R. simus*—and that only in the somewhat loose sense in which the term is applicable to varieties of *Rhipicephalus*. Now *R. lunulatus* ♂ is in all respects a somewhat small *R. simus* except for its very striking anal plates, but unfortunately these grade into each other absolutely, and, moreover, the projections which give to the anal plates of *R. lunulatus* their very distinctive facies are

often transparent and give the effect of being superposed on the anal plates proper to *R. simus*. The females also, of which we possess several specimens, differ from a typical *R. simus* ♀ in having their scuta usually more angular and more punctate, but the females of undoubted *R. simus* differ considerably in this latter respect.

The *lunulatus* form of *R. simus* occurs frequently on large mammals in Nyasaland. The ♂ type was from a horse in the Congo Free State, near the river Lualaba.

To recapitulate, *R. lunulatus* must be degraded to a variety of *R. simus*. *R. glyphis* lapses to a synonym of *R. simus* var. *lunulatus*.

R. longus Neumann, 1907.

The single type specimen (a male) of this species proves, on careful examination, to be a somewhat ill-characterised example of *R. falcatus*. Among the numerous specimens of *R. falcatus* possessed by us there are several specimens which match it precisely. *R. longus* (type at Cambridge) therefore lapses and becomes a synonym of *R. falcatus*.

R. supertritus and R. coriaceus.

The types of *R. supertritus* Neumann, 1907 in the British Museum are identical with the form described by Nuttall and Warburton (N. and W. 1907) as *R. coriaceus*.

The descriptions both bear the date 1907, but as that of *R. coriaceus* was only published on Dec. 28, *R. supertritus* doubtless has priority. *R. coriaceus*, therefore, becomes a synonym of *R. supertritus*.

REFERENCES.

The references are to the "Bibliography of the Ixodoidea" in *Ticks, a Monograph of the Ixodoidea*, Cambridge University Press, 1911.

TRYPANOSOMES FOUND IN CANADIAN MAMMALS.

By E. A. WATSON, V.S., and S. HADWEN, D.V.Sci.
Department of Agriculture, Canada.

(Plates I and II.)

Since 1906 trypanosomes have been found in ten species of mammals by officers of the Health of Animals Branch.

Only one of these parasites has been proved to be pathogenic, *i.e.* that of Dourine.

As will be seen by referring to the plates, several of the apparently non-pathogenic forms bear some resemblance to those trypanosomes which are well known to produce fatal diseases.

Other Canadian mammals doubtless harbour trypanosomes and further species will be recorded.

Intense cold seems to have little effect on the range of these parasites, and it is interesting to note that virulent outbreaks of Dourine have occurred in places where the thermometer sometimes drops to 50° and 60° F. below zero. However, the identity or the non-identity of this trypanosome with that of African Dourine remains open to question, and, on similar grounds, the identity of the African with the so-called Indian and European varieties may well be doubted.

The Dourine of Canada appears to be identical with Beschälseuche of East Prussia, the Dourine of Hungary and that of Eastern Europe. In any of these, the trypanosome is rarely, if ever, found in the general circulation of infected animals, but only in the serous or sanguineous fluids of local swellings and infiltrations which are characteristic of the malady; further, they closely correspond in that laboratory animals are

notoriously resistant to them, and there are but very few instances recorded of successful infection, in a rat or rabbit, and no investigator seems to have been able to maintain a laboratory strain for any length of time. These trypanosomes, therefore, show a remarkable difference, biologically, from the parasite of Algerian or African Dourine, which is said to be observable in the general circulation of infected horses, and has been transmitted to and carried along in dogs, rabbits, rats, mice, etc. without difficulty, becoming exceedingly virulent for laboratory animals.

Trypanosoma leporis-sylvaticus n. sp. Watson.

(Pl. I. fig. 1.)

Found in the "cotton-tail bush rabbit," *Lepus sylvaticus*, at Lethbridge, Alberta, Nov. 17, 1906. (Watson.)

This trypanosome has been observed in about 30 per cent. of rabbits shot or trapped at the Lethbridge Experimental Station, 1906–1911. Blood infection may be noted at any season of the year but the parasites are present in greater numbers and in a greater percentage of animals during the late fall and early winter months than at any other time. They usually disappear from the blood of rabbits held in captivity after a few days, but occasionally persist for a month or longer period.

The trypanosome is 26·7 μ in average length; it differs from the well-known *lewisi* type in being more slender and elongated, in having a more pronounced undulating membrane, a more centrally situated trophonucleus, and a larger and more rounded kinetonucleus.

This trypanosome is apparently non-pathogenic for native and domesticated rabbits, mice, and pigeons. (Watson.)

Trypanosoma peromysci n. sp. Watson.

(Pl. I. figs. 2, 3.)

Found in northern deer-mice, *Peromyscus maniculatus*, *P. nebracensis*, and other species, Lethbridge, Alberta, Dec. 1906. (Watson.)

About 20 per cent. of these mice are found infected. The seasonal prevalence is similar to that of the rabbit trypanosome. The parasites disappear from the blood of mice held in captivity usually on the second or third day and have never been seen after a period of seven days.

The average length of the trypanosome is 28 μ. The trophonucleus

is not so centrally located as in that of the rabbit trypanosome nor as far forward as in *T. lewisi*, and the posterior end of the parasite is narrower than in either of the other two species.

Non-pathogenetic for mice and rabbits. (Watson.)

Trypanosoma equiperdum Doflein, 1901.

(Pl. I. fig. 4.)

The first discovery of the Dourine trypanosome in North America was made at Lethbridge, Alberta, on Feb. 11, 1907, in a naturally infected mare. (Watson and Gallivan.)

Seasonal prevalence: trypanosome periodicity has been noted in horses in every month of the year, not infrequently during the coldest winter months, but the parasite becomes most active, usually, towards the end of a very hot summer season.

Pathogenicity: different strains of Dourine trypanosomes have been found to vary greatly in virulence. The strain isolated in Feb. 1907, became exceedingly virulent for horses after eight or nine early passages through young mares and foals.

Dogs, rabbits, mice and gophers were always resistant; white rats were less so, for after a great number of failures to infect with strains of ordinary virulence, a few of these animals were at last successfully infected with the strain which had become so highly virulent for horses, and although the parasites were never seen in the rats' blood, this blood, when injected into horses produced a virulent and fatal infection.

Morphological characteristics: the average length of the parasite is 27 μ. The anterior extremity has a free flagellum, usually of short length; the posterior is frequently blunted or has a sawn-off appearance. The kinetonucleus is very small, smaller than in any of the pathogenic trypanosomes with the exception of *Trypanosoma equinum*, and is frequently associated with, or just posterior to, a vacuole. (Watson.)

"Dourine in Canada." J. G. Rutherford. *The Lancet*, May, 1907.

Special Report on Dourine. *Health of Animals Branch*, Dept. of Agriculture, Canada. Nov. 1907[1].

"Note on the life-history of *Trypanosoma equiperdum*." E. A. Watson, in *Health of Animals Report*, 1909[1].

"An Experimental Study of Dourine." E. A. Watson, in *Health of Animals Report*, 1910[1].

[1] Published by the Department of Agriculture, Ottawa, Canada.

Trypanosoma citelli n. sp. Watson.

(Pl. I. figs. 5, 6.)

Found in the ground-squirrel or prairie-gopher, *Citellus richardsoni* (Sabine), at Lethbridge, Alberta, on April 5, 1908. (Watson.)

The blood of three ground-squirrels out of 12 examined (March—July, 1908) showed trypanosomes. Unlike the rabbit and mouse infections, in which the parasites are often plentiful, only one or two parasites could be found in each slide preparation of blood of infected animals.

This trypanosome is 35 μ in length and has morphological characters that differentiate it from any other species recorded in this paper. Excepting the giant trypanosome of the cow, it is considerably longer and the body of the parasite terminates anteriorly more abruptly, leaving a long free flagellum. The posterior extremity is very slender and finely pointed, the trophonucleus is elongated and well forward, and the kinetonucleus always appears round, never elliptical or rod-shaped.

Non-pathogenic for gophers, mice and rabbits.

Trypanosoma lewisi (Kent), 1882.

Found in six rats out of 16 examined at Ottawa, Ontario, on 24.1.07. (Hadwen.)

Trypanosoma sp. Bowhill, 1909.

From squirrel's blood. Mount Lehman, B. C., Fig. 31, in *Health of Animals Report*, 1909. (Bowhill.)

Trypanoplasma sp. Bowhill, 1909.

In blood of cow. Mount Lehman, B. C., Fig. 29. "Red water' investigations in British Columbia" in *Health of Animals Report*, 1909. (Bowhill.)

No description is given of this parasite; it is probably a large trypanosome instead of a trypanoplasm and may be identical with *T. rutherfordi*, described below.

Trypanosoma rutherfordi n. sp. Hadwen.

(Pl. II. fig. 10.)

A single parasite was found in the blood of a cow at Mount Lehman, B. C., on 18.4.10. (Hadwen.)

The parasite appears to be non-pathogenic as the cow was fattened later and killed for beef. A rabbit was inoculated with blood but

suffered no ill effects. This giant trypanosome, which measures 55 μ in length, may possibly belong to the *theileri* group. The body of the organism has a broad or stumpy appearance and anteriorly is not finely drawn out as in *T. theileri*, but, on the contrary, terminates somewhat abruptly and has only a very short free flagellum. Posteriorly, a slender filament is extruded from the rounded end of the body. The nucleus is situated posterior to the centre, the endoplasm appears coarsely granular and is profusely vacuolated. No doubt this is an old form of the parasite and probably is at the commencement of degeneration.

Trypanosoma evotomys n. sp. Hadwen.

(Pl. II. fig. 7.)

Found in a vole, *Evotomys saturatus* (Rhoads) at Mount Lehman, B. C., on 27.7.11. (Hadwen.)

A long parasite, average length 26·5 μ, differing from *T. lewisi* in having the nucleus close to the centre, and in possessing a well-developed undulating membrane and a much larger centrosome. The distance between centrosome and nucleus is much less than in *T. leporis-sylvaticus*, *T. peromysci*, and *T. citelli* (compare with figs. 1, 2, 3, 5 and 6).

Found in two mice out of ten examined.

Non-pathogenic for two rabbits inoculated 27.7.11, still alive on 10.10.11. (Hadwen.) Also non-pathogenic for guinea-pigs. (Dr McKee.)

Trypanosoma soricis n. sp. Hadwen.

(Pl. II. figs. 8, 9.)

Found in blood of wandering shrew, *Sorex vagrans*[1] (Baird) at Mount Lehman, B. C., on 28.7.11. (Hadwen.)

A short active parasite with the nucleus in centre, undulating membrane well marked, and a very short free flagellum. The organism is broad and stumpy and the total length only 17·5 μ. Found in two out of five mice examined. Apparently non-pathogenic.

We are indebted to Dr J. C. Rutherford, C.M.G., Veterinary Director General, for permission to publish these notes.

[1] These mice were kindly identified by F. Kermode, Curator of the Provincial Museum, Victoria, B. C.

EXPLANATION OF PLATES I AND II.

	Species	Average length	Host
Fig. 1.	*T. leporis-sylvaticus* n. sp. Watson.	26·7 μ	Rabbit, *Lepus sylvaticus*.
Figs. 2 & 3.	*T. peromysci* n. sp. Watson.	28 μ	Mice, *Peromyscus maniculatus*, *P. nebracensis*.
Fig. 4.	*T. equiperdum* Doflein, 1901.	27 μ	Horse.
Figs. 5 & 6.	*T. citelli* n. sp. Watson.	35 μ	Ground-squirrel, *Citellus richardsoni*.
Fig. 7.	*T. evotomys* n. sp. Hadwen.	26·5 μ	Vole, *Evotomys saturatus*.
Figs. 8 & 9.	*T. soricis* n. sp. Hadwen.	17·5 μ	Shrew, *Sorex vagrans*.
Fig. 10.	*T. rutherfordi* n. sp. Hadwen.	55 μ	Cow.

Fig. 1.

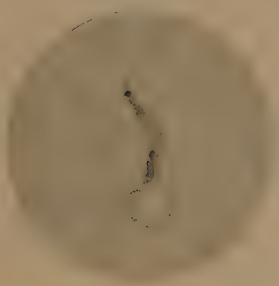

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

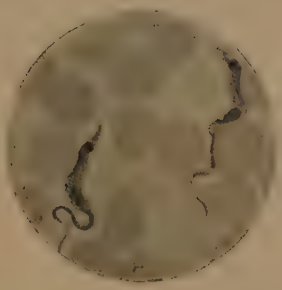

Fig. 7.

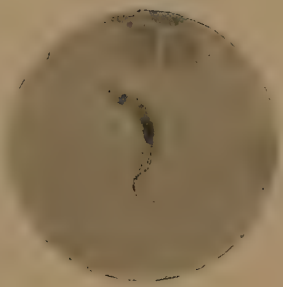

Fig. 8.

Fig. 9.

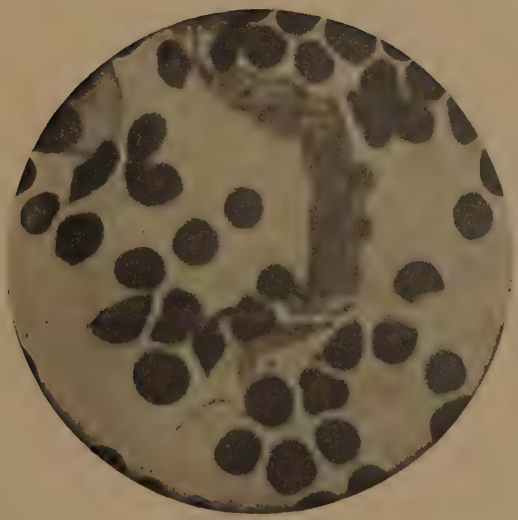

Fig. 10.

THE CEYLON PEARL INDUCING WORM.

A BRIEF REVIEW OF THE WORK DONE TO DATE.

By T. SOUTHWELL, A.R.C.Sc. (Lond.), F.L.S., F.Z.S.
Deputy Director of Fisheries for Bengal. Late Scientific Adviser and Inspector of Pearl Banks to The Ceylon Company of Pearl Fishers Limited.

I propose in this paper reviewing very briefly the work which has been done on the Ceylon pearl-inducing worm, during the last ten years. This is particularly appropriate at the present time as the Pearl Banks—which have been under lease to a London Syndicate, who have spared no pains or money in the investigation—may at an early date revert back to the Government. It remains to be seen to what extent the Ceylon Government will profit by these investigations, and whether they will continue the work with the vigour which has characterised the Company's administration.

Most people are familiar with the ancient and poetical beliefs regarding pearls, viz. that they were the tears of Nereids, or consolidated drops of dew, or caused by lightning flashes. Later on these romantic beliefs were superseded by others which attributed the origin of pearls to the irritating effect caused by the presence of grains of sand, and other foreign bodies, in the tissues of the oyster.

Kelaart, as a result of a few years work on the spot, about the year 1858, observed that pearls were intimately connected with the presence of "worms" in the oyster, and in 1894 Thurston confirmed Kelaart's results and identified the worm as the larva of a Platyhelminth. These results represent all that was actually known regarding the pearl-inducing worm up to the year 1902.

About this time the intermittent nature of the Pearl Fisheries seriously occupied the attention of the Colonial Government, as these

fisheries were a considerable but intermittent source of revenue. The Royal Society was approached to send a Zoologist to investigate the Pearl Fishery problems and, as a result, Professor Herdman arrived in Ceylon in 1902. The discovery of the pearl-inducing worm dates from that time. Large collections of Cestode parasites from marine fishes were made. These were described by Dr Shipley and Mr Hornell (1). The larvae inhabiting the tissues of the oyster were identified as Cestode larvae, and the adult worm which was named *Tetrarhynchus unionifactor* was found in *Rhinoptera javanica.*

Cysts of this worm were also obtained from certain species of fish of the genus *Balistes* but the position of these cysts with relation to the life history of the parasite itself was left in some doubt. In 1905, the banks were leased to the Ceylon Company of Pearl Fishers Limited, and the work done since that time will be detailed in this paper.

Two kinds of pearls occur in the Ceylon pearl oyster. Those pearls which are formed round the larvae of *Tetrarhynchus unionifactor*—and very occasionally round other nuclei—are termed "cyst" pearls. They are usually round, and if old, are of considerable size. The second kind of pearls have a totally different origin. They almost always occur at the insertions of the levator and pallial muscles. They are termed "seed" or "muscle" pearls. They appear to have no nucleus and they are always very small and of irregular shape. Their origin is unknown. By some they are considered to be excretions caused by irritation set up consequent on the "shear" of muscles working in different planes. By others they are considered to be excretions due to a superfluity of lime in the organism. No nuclei have as yet been discovered in them, but they are believed by some to be formed round a primary limey nucleus which has been termed a calcospherule.

The early stages of the life history of the pearl-inducing worm are not known. Herdman recorded the presence of free-swimming Cestode larvae in the plankton collected on the Pearl Banks, but as he mentioned later "it is still uncertain whether the free-swimming larvae found on the Muttuvaratu Paar really belong to the life history." Although the plankton has since been collected fairly regularly over the pearl banks, no Cestode larvae have been identified, and it would be obviously impossible at the present stage of our knowledge to identify an adult worm from larvae so young.

Nothing is known as to how the larvae enter the oyster. The adult worm occurs in fish which live on the bottom and are bottom feeders.

Under these circumstances it is easy to imagine that the ripe proglottides drop in the close vicinity of the oysters on which the fish concerned normally feed. But we do not yet know whether the larvae are free-swimming, and bore their way into the oyster, or whether they are passive, and depend for their development on being ingested as food. So far as is known only the larvae of the *Bothriocephalidae* are ciliated and free-swimming, although it may be possible that many Cestode larvae are free-swimming without being ciliated.

Our knowledge however of the earliest stages of the vast majority of Cestodes is practically *nil*. As illustrating this point I may here note that some time ago, whilst examining the ripe proglottides of a specimen of *Tetrarhynchus rubromaculatus*, I found that the segmenting eggs, issuing in immense numbers from a ruptured proglottid, were ciliated, a phenomenon I have not seen noted elsewhere.

In view of the microscopic nature of the eggs of *Tetrarhynchus unionifactor*, the chances of ever discovering the actual method of entry of the larvae into the oyster itself, are remote indeed. Our first knowledge then of the pearl-inducing worm begins with the larvae found in the tissues of the oyster. These larvae are globular, and may be found in any part of the tissues. Figures of this larva will be found in Herdman's *Ceylon Reports* (Shipley and Hornell). They vary in size from 1 mm. to tiny larvae which can only be seen under a magnification of 120. All sizes are to be found between these extremes. The presence of these very varying sizes of larvae in the oyster at first gave rise to the impression that they represented larvae of different species, possibly of the same genus. Subsequent investigation however brought to light a different explanation (Southwell 1909–1910). It was observed that the larvae multiplied endogenously by the production of daughter cysts internally which escape by the temporary rupture of the parental wall. Thus although the initial infection of the oyster may be only slight, it usually becomes extensive merely by this method of endogenous reproduction, independent of a further direct infection. This method of multiplication fully and naturally accounts for the very varying sizes of larvae found in the oyster. At present this form of reproduction has only been observed to be monogenetic, that is one daughter cyst only is born at a time, but it may be found later to be digenetic, or polygenetic. Willey (1907) observed a similar phenomenon in the larvae inhabiting *Placuna placenta* (the window oyster) and in this case the reproduction of daughter cysts was polygenetic. It is now tolerably certain that the Cestode larvae found in the tissues

of *Placuna* are the same as those found in the Ceylon pearl oyster. We shall however return to this subject later. Haswell and Hill (1894) also recorded a similar case of endogenous multiplication in *Polycercus* —the bladder stage of *Taenia nilotica* from *Cursorius europaeus.*

This globular cyst then, found in the pearl oyster, is the first known stage in the life history of the pearl-inducing worm. It is round these larvae that cyst pearls are formed. The remains of the larva have been identified in the centre of sections of decalcified pearls. It appears that although cyst pearls almost invariably originate in this way, other nuclei may very occasionally lead to pearl formation. Hornell records two cases in which grains of sand have been found to act as nuclei.

The outstanding feature in connexion with these larvae, and their relation to pearl formation, is, that so few pearls are found, although the larvae are so abundant. A normal oyster may contain as many as 200 larvae and not a single pearl may be present. One is well within the mark in stating that not 0·001 of the larvae present in the Pearl oyster ever become pearl nuclei. It seems likely that cyst pearls are only formed round such parasites as for some unaccountable reason die in the tissues of the oyster and thus set up local irritation, resulting in a migration of ectodermal cells which eventually cover the irritating particle with pearly matter. This pearly matter is secreted round the parasite in layers, simulating the underground leaves of an onion.

It would appear possible, and even likely, that future scientific investigation will be directed towards enquiring more fully into this question and, should it be found possible to treat oysters in such a way that a large percentage of these larvae become pearl nuclei, pearl fishing in Ceylon will be revolutionised. A few experiments of this kind have been carried out on the Ceylon Pearl Banks but with only a little success. The operations were severely hampered by the almost complete absence of oysters.

Up to the present, all the methods of culture in Ceylon have been directed towards the preservation of *beds* of oysters, and no attempts have been made to enhance the pearl-yield of the individual oyster. It is obvious that the pearl-yield bears some relation to the degree of infection, but instead of leaving the results to nature, something may be done in the future to assist nature in the way indicated.

A form of pearl culture is extensively carried on in Japan, which consists of the introduction of small leaden images of the Buddha or other nuclei between the mantle and the shell. In a short time these nuclei

become covered with concretions which adhere to the shell. When these concretions have reached a suitable size, they are carved out of the shell and sold. The industry is carried on largely by women who have small farms of about 1,000,000 oysters. The pearls thus artificially produced are never round and they always have one bad face, and are therefore only used for mounting, when the defects are hidden in the mount. It is obvious that if the potential pearl-inducing larvae of the Ceylon oyster could be treated so as to die *in situ*, the production of true cyst pearls would be greatly enhanced and would result in the fishing of a small number of these oysters being as profitable as is the fishing of millions at the present time.

Passing on from this first known stage in the history of the pearl-inducing worm (viz. the globular cyst in the oyster), we find that the next stage known is the young but adult Tetrarhynchid occasionally found encysted in the gut of the oyster. Being practically adult, they are so different from the globular cyst that there seemed at first to be no particular reason for presuming that they represented different stages in the development of the same parasite. They occur almost in every case encysted in the wall of the gut, just where the intestine turns to run direct to the anus. They are usually found in clusters of two to five. They often measure 1·5 mm. The four proboscides and the two bothridia are fully formed, and the former are often protruded. No strobila is present, but the worms are adult in every other way, except that they are small. The dissimilarity between this stage and the stage represented by the globular cyst will be obvious when it is stated that the larvae in the globular cyst are so young that the Cestode characters are by no means well defined. No stage or stages have ever been found intermediate between them, and the evidence that they really are stages in the life history of the same parasite rests on circumstantial evidence only and on the results obtained by the feeding experiments to which we shall refer later. The only other known stage in the life history of this parasite is the adult worm itself, which was first obtained from *Rhinoptera javanica* It is unfortunate that specimens are so rare that the adult has not been described as fully as it might have been. With the material now in my possession, and the observations which have been made, I am hoping shortly to add further particulars regarding this parasite. So rare is the adult, that, in spite of the fact that several thousands of fish, caught with the trawl, have been carefully and repeatedly examined—including large numbers of both sharks and rays—the adult has never since been found except in *Ginglymostoma*

concolor, during the feeding experiment of 1909 and 1910, and described in Part IV and V, *Ceylon Marine Biological Reports*.

This is a very remarkable fact, and is no doubt to be correlated with the absence of oysters from the banks during the past five years, and to the resulting general scantiness of fish over the plateau. Up to the present, about 105 species of Cestodes have been recorded from fish caught on the pearl banks, and as the adult of the pearl-inducing worm was never obtained, its rarity will be obvious.

We have already noted that no larvae have ever been found in an earlier stage of development than the globular cyst; that no stage has been found intermediate between the globular cyst and the adult but young Tetrarhynchid both of which occur in the oyster; and finally we have noted how very rarely the adult worm itself is found.

In 1909 experiments were initiated with a view to develop the adult worm in various Plagiostomous fish by feeding them with oysters containing the globular cysts. These experiments are fully described in the Reports above cited. A square area in the open sea was isolated by means of expanded metal placed vertically. The fish deemed suitable for experiment were often trawled 10 to 12 miles away and were transported to the enclosure in the ship's water boat, which had a mid-ship tank section of $13 \times 9 \times 4\frac{1}{2}$ feet which could be flooded with sea water, and used to contain the fish. The bottom of the enclosure was specially covered with oysters of various ages. Before the fish were placed here they were dosed with male-fern extract and castor oil, in order to clear away, if possible, any parasites they might already have, before the experiment was begun. The general success of this procedure was indicated after the ultimate death of the fish by the large number of reddish spots in the spiral valves, indicating the positions previously occupied by Cestodes. The following fish were thus dosed and then placed in the enclosure: *Trygon walga, Taeniura melanospilos, Gingly-mostoma concolor, Rhynchobatus djeddensis, Serranus undulosus* (4 feet long) and specimens of *Tetrodon stellatus* measuring 20 inches long. The results were roughly as follows:

(1) Although several specimens of *Rhynchobatus djeddensis* were placed in the enclosure they all died within three days, showing that in spite of their massive teeth and strong jaws this species will starve to death rather than eat oysters.

(2) Specimens of *Tetrodon stellatus* and *Serranus undulosus* lived in a healthy condition, but on being killed after several weeks no adult Cestodes were found in them.

(3) *Taeniura melanospilos.* Adult specimens of *Tetrarhynchus herdmani* only, found in the spiral valve. This fish was an enormous specimen measuring seven feet six inches.

(4) *Ginglymostoma concolor.* Adductor muscles of oysters found in stomach. Thirty-eight specimens of *Tetrarhynchus unionifactor* in one specimen (1910) after 31 days in the enclosure, and fifty-one specimens in another (1909) which had lived in the enclosure 28 days.

These results are fully described in Parts IV and V, Ceylon Marine Biological Reports. The point that immediately concerns us, is, that of the fish tried the only one suitable for the experiment was *Ginglymostoma concolor.* That the various fish placed in the enclosure had been eating oysters vigorously, was witnessed by the fact that the bottom of the enclosure was littered with fragments of broken shell. I was enabled to actually see the extent of these depredations by means of the diving dress. Although specimens of this fish had previously been examined no specimens of *Tetrarhynchus unionifactor* had ever been obtained from them. It is therefore almost certain that these Cestodes were derived from the oysters eaten. Otherwise it would be difficult to account for their presence in this fish on two successive occasions, and their absence from the same species of fish—as in every other species of fish examined—direct from the open sea.

It is further to be remembered that during the time the experiments were being conducted, oysters were practically absent from the banks, and the only specimens obtainable were scattered specimens found growing on reefs, and in which pearl-inducing larvae were exceedingly rare. I have no doubt in my own mind that if a similar experiment be repeated when oysters are common on the banks, the results will be made absolutely conclusive. One seems justified therefore in saying that these specimens of *Tetrarhynchus unionifactor* were derived from the larvae inhabiting the tissues of the oysters eaten, and that the life history of the parasite is direct from the oyster to the final host.

During 1907 I had numerous opportunities of examining the pearl-inducing larvae found in *Placuna placenta* (the window oyster) occurring in the backwaters at Trincomalee on the east side of Ceylon. This larva is exactly similar to that of the pearl-inducing worm of the pearl oyster. As we have already noted, Willey pointed out that this larva, found in *Placuna*, multiplied in an endogenous manner. During a visit to Tamblegam in September, 1911 I examined a few specimens of

Trygon spp. which I obtained from local fishermen, and in one species found forthwith a few specimens of *Tetrarhynchus unionifactor*. This circumstance lends support to the belief that the pearl-inducing larvae inhabiting these far removed genera of pearl bearers are the same as those found in the pearl oyster. In view of the close proximity of these oysters this fact is not surprising. The astonishing fact is that the adult worm should apparently be so common at Tamblegam and so rare on the Pearl Banks.

There seems to be no reason for presuming that the adult of the pearl-inducing worm in the pearl oyster occurs exclusively in *Rhinoptera javanica*. We have already shown that it also occurs in *Ginglymostoma concolor* and in *Trygon* sp. and there seems every reason to believe that future investigations will prove its presence in all Plagiostomes which feed on oysters. I know of no Cestode obtained from the Pearl Banks which does not occur in more than one host, and in many instances the adult worms are found in as many as seven species of fish. Moreover it is natural to presume that the adult worm will occur in species which normally eat oysters.

It has already been pointed out that many Teleosts feed on oysters, such as members of the genera *Balistes*, *Serranus*, *Lutjanus*, *Tetrodon*, and genera allied to the latter. Except in the genera *Tetrodon*, *Tetrarhynchid* cysts have been found on the mesenteries of nearly all the specimens examined of the above genera, and this fact originally led to the idea that possibly members of the genus *Balistes* (which were better known at that time than the other genera) formed an intermediate host for the pearl-inducing parasite. Shipley and Hornell however pointed out that the young adult larvae found encysted in the gut of the oyster were different from those obtained from *Balistes*. This fact has been amply corroborated since, but careful search has revealed the fact that encysted forms of *Tetrarhynchus unionifactor* do occur both in certain species of *Balistes* and *Serranus*, and the young adult found encysted in the mesenteries of certain of these genera is identical in every way to the young adult found encysted in the gut of the oyster. It is difficult to understand why it is that in the oyster itself two stages occur in the development of the parasite which are so widely separated. True, the young adults are very rare as compared with the globular cysts. We are not *absolutely* certain that these two larvae belong to the same parasite, although the circumstantial evidence afforded by the feeding experiment seems to indicate that they are. Moreover, if the adult specimens of *Tetrarhynchus unionifactor* obtained

during the feeding experiments were developed from the globular cysts in the oyster, then the young adult also found in the oyster must be the same species, for it corresponds in every detail with the adult, except in size. The position of these cysts containing young adults of *Tetrarhynchus unionifactor*, found in *Balistes* and *Serranus*, with reference to the life history of the parasite is not quite easy to understand. No adult Cestodes have ever been obtained from any Teleost caught on the Pearl Banks, and although this fact is very surprising, it is not unique, but falls in line with the observations made by others in different parts of the world. These bony fish are to be regarded as collateral, but not as intermediate hosts. When oysters are eaten by them, the globular cysts derived from the oyster develop, in the bony fish, into young adults, but no further. The strobila is never developed, and the stage attained is no further advanced than (in fact it is the same as) the young adults occasionally found encysted in the gut of the oyster itself. The stage in *Balistes* and *Serranus* represents a cul-de-sac in the life history of the parasite, for most of the species of these genera of fish are of such large size, that it is difficult to postulate a Plagiostomous host large enough to devour fish of such dimensions. In these cases we can only assume that the life cycle of the parasite is never completed, a circumstance homologous with the occurrence of hydatids in man, where the larvae giving rise to the disease have, owing to their capability of adaptation within various hosts, lost themselves in the maze of their own liberties, and where of course the life history is never completed. These genera of fishes cannot therefore be considered as intermediate hosts. They are collateral hosts to the larvae.

One assumes that should one of these Teleosts be devoured by a Plagiostome the encysted form of *Tetrarhynchus unionifactor* would develop into adult, just in the same way and to the same extent that the two types of larvae in the oyster do. Unlike certain Plagiostomes, where the larvae develop into the adult and liberate myriads of eggs, these Teleosts merely devour oysters without extending the means of infection. For oyster culture these Teleosts are undesirable in every way.

Although I believe that this represents the real state of affairs existing between the oyster, *Balistes* and *Serranus*, and the final host of the adult parasite, it may be that in the case of other parasites, bony fish really act as intermediate hosts, in which case the parasite in question would have three hosts. In other cases it may be found that the smaller Teleosts are initially infected, and that the adult occurs in the

Plagiostome devouring them, in which case only two hosts would be present. In view of what appears to be the case with *Tetrarhynchus unionifactor*, it seems more likely that Cestodes in general have only two real and necessary hosts, and that the stages found in these bony fish merely extend the distribution of the larvae without being essential —or in many cases useful—for the fulfilment of the life history.

It is astonishing how little is known of the life history of Cestodes in general. Of the 105 species collected from the Pearl Banks the life history of *Tetrarhynchus unionifactor* alone is known, and this not completely.

Moreover, innumerable encysted forms of Cestode larvae are to be found in the molluscs, crabs and bony fish inhabiting the Pearl Banks, but the adults are not known.

In the elucidation of these problems alone, there lies ample scope for future work.

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THE DEVELOPMENT OF *LEUCOCYTOZOON CANIS* IN THE TICK WITH A REFERENCE TO THE DEVELOPMENT OF *PIROPLASMA*.

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(With 2 Diagrams.)

In the last issue of *Parasitology* a description is given by Wenyon (5) of the developmental cycle of *Leucocytozoon canis* in the tick *Rhipicephalus sanguineus*. Wenyon refers to the description of the development given by me (2), but rightly points out that one stage, that of the large compound cysts, was overlooked. The surmise, however, that these large and easily ruptured cysts were overlooked owing to their having been broken up in the preparation of films is only part of the truth. A full explanation involves facts in regard to the development of this parasite which have not been touched upon by Wenyon; it is possible, as will be seen shortly, that Wenyon's description of the cycle, like mine, involved a considerable error. In this respect, as a result of work not yet published, I am able to supplement what has been written on the subject.

But little stress has been laid by Wenyon on the possibility that the forms supposed to be developmental forms might be merely stages in the development of some natural parasite of the tick. In this respect I shall also give the result of observations made since my first description of this cycle.

Development of the parasite in relation to the growth of the tick.

In a previous publication I described the following stages of development in the body of the tick:

1. The liberation of motile vermicules and the passage of these into cells of the gut (*diverticulum*) wall.

2. Fission of the original vermicules.

3. Conjugation of two apparently similar vermicules, the result of this fission.

4. The formation, as a result of conjugation, of a rapidly growing *oocyst* and certain other details described in the paper.

5. Growth of the oocyst.

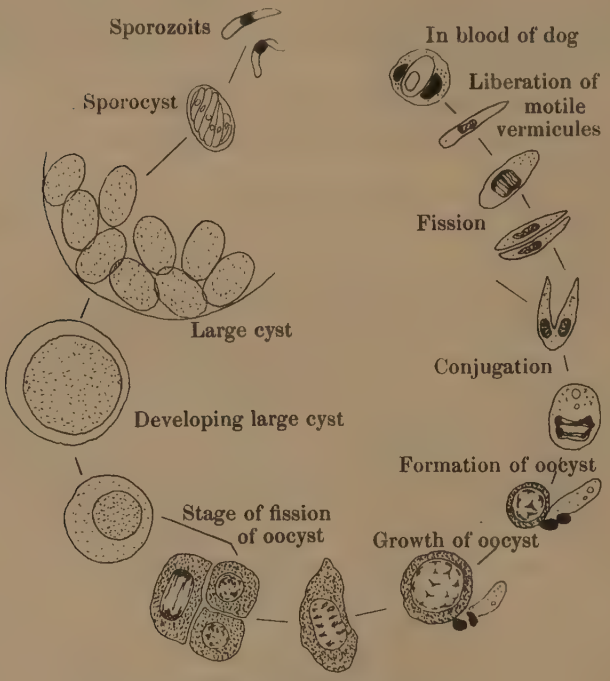

Diagram 1. Showing the developmental changes of *Leucocytozoon canis* in the tick.

6. A change in the oocyst by which after reaching a certain size it loses its globular shape and becomes an irregularly shaped body as depicted in the plate accompanying the paper. (Fig. 25.)

7. The formation of groups of sporozoites, as I then thought from the further development of the oocyst.

Further work has shown that I was correct with the exception of the step from 6 to 7.

The error in this step was not merely that of missing the large cysts since described by Wenyon. Just as I believe Wenyon has done, I here short circuited the developmental cycle by describing stages which were not the result of development of parasites taken in by the adult tick at all, but were the last stages of a development undergone by a previous brood of parasites taken in by the tick in its nymphal stage.

To continue the story of development one may return to the large deeply staining irregular bodies which are the latest stage I have correctly described as following the ingestion by the adult tick of blood containing haemogregarines. After these bodies have grown somewhat in size, the nucleus exhibits karyokinetic changes and, strange as such a fact may seem, the oocyst (?) divides first into two and then into four. I have repeatedly confirmed the occurrence of this act of division, and the groups of two or four large deeply staining bodies the result of fission occur quite regularly and form a conspicuous feature in development at a certain stage. Later development consists in a great increase in size of these forms, which, however, as they increase in size begin to take the stain less and less deeply. The chromatin also which at first stains readily becomes in the later stages more difficult to stain until it is clear that one is dealing with a cyst wall. By the time these bodies have reached 40 μ or so in diameter they are demonstrably the early stages of the large cysts described by Wenyon and can readily be picked out under a low power.

Wenyon's account suggests that the large cysts found by him lying *outside* the gut wall were the result of development of parasites taken in when the adult tick had been fed. But in my own observations, when an adult tick was fed on blood containing haemogregarines, these required so long a period to develop that, even when the tick had shrivelled after the lengthy process of oviposition, the large cysts were only partially mature. In ticks kept for 24 days (in the Madras climate) most of the cysts were still immature and still later many had become stained with blood-derived pigment and were seemingly undergoing degeneration. *Also all the cysts thus formed lay loosely amongst the products of digestion in the lumen of the gut.*

When a tick was fed as a nymph upon an infected dog the same sequence of developmental changes took place[1], but the large cysts were

[1] In my original description of the cycle I recorded two experiments in which parasites had disappeared by the fourth day from nymphs fed on infected blood. Later experiments have shown that, on the contrary, development proceeds very rapidly in the nymph. Possibly I was misled by this fact and overlooked the oocysts in my early experiments.

already well advanced in development by the time the adult had hatched out from the pupa-like nymphal resting stage. Before the tick has fed the cysts are found lying in the lumen of the, as yet, narrow and empty diverticula, distending these much as a tennis ball might distend a stocking into which it had been pushed. But when the tick has attached itself and the diverticula become enormously swollen with blood, the cysts, which are still increasing in size, are pushed aside and come to lie in little pouches formed of the membranes of the gut wall: they then appear in dissection as if attached by a delicate pedicle to the outer wall of the gut. *Large cysts lying outside the walls of the gut are therefore evidence of infection during the nymphal stage and have nothing to do with the development of vermicules taken in by the adult.* In spite of this evident error of interpretation, however, the actual cycle described by Wenyon is correct and corresponds with that described by Miller (4) for *H. perniciosum* in the mite (*Lelaps*).

The later stages of development are only seen in ticks which have been infected during the nymphal stages. During the stage of engorgement the large cysts develop rapidly and become fully mature and some may by rupture of the pouch in which they lie be set free in the body cavity of the tick. In a heavy infection these free cysts tend to collect together in the neighbourhood of the base of the rostrum, and some evidently are ruptured since the smaller sporocysts containing sporozoites are found free in the body juices. Since my original note was written I have examined numbers of very heavily infected ticks, some of them kept until they had become greatly shrivelled after oviposition, but I have never succeeded in finding sporozoites in the salivary glands or ovaries.

In my first note (2) I suggested that infection might occur by the dog swallowing the gorged adult tick and Miller has actually produced infection in this way with *H. perniciosum* by feeding white rats on infected mites. Unfortunately such an experiment has not yet been carried out in the case of *L. canis*.

There is, however, another way in which infection may occur. In adults infected as nymphs I have on several occasions found numerous sporozoites *within* the gut. My work requires confirmation in this important point, but I have found them even when portions of the diverticula have been removed and washed, and when careful search with a lens showed that there were no adherent cysts. The presence of sporozoites in the lumen of the gut may be due to these having repenetrated the gut wall, or to cysts rupturing whilst still lying in

pouches of the diverticulum wall. In this latter case the sporocysts would easily pass through the neck of the pouch into the lumen of the diverticulum. If sporozoites, the result of a previous nymphal infection, occur free in the gut of the adult tick during the act of engorgement, it would not be very difficult for them to pass at this time into the body of the second host.

Development of other mammalian haemogregarines in arthropod hosts.

In my description of *H. gerbilli*, a haemogregarine found in the red blood corpuscles of the common Indian Jerboa Rat (*Gerbillus indicus*), I described what I took to be developmental stages of this parasite in the louse (*Haematopinus stephensi*) commonly found infesting these animals, *i.e.* free vermicules in the gut and large cysts containing numerous sporocysts in the body cavity. For several reasons, notably Patton's failure to find similar cysts in lice fed on squirrels infected with *L. funambuli*, I thought later that I might have fallen into one of the frequent errors connected with the tracing of the cycles of development in supposed or known carriers. But the large cysts in this case are indistinguishable from those associated with the development of *L. canis* in the tick, and with those described by Miller in that of *H. perniciosum* in the mite. This fact, taken in conjunction with the results of dissections of lice from infected and uninfected gerbilli given in my paper, makes it more than probable that I had correctly described these cysts as a portion of the extra-corporeal cycle of the haemogregarine in question.

Following my description of the cysts in *Haematopinus*, Balfour (5) noted similar large cysts in the case of a flea (*P. cleopatrae*), caught on jerboas infected with *H. jaculi*, also a haemogregarine attacking the red corpuscles of the host. Under the impression that the cysts described by me were not a stage in the development of *H. gerbilli*, as first described, he failed, except at first, to attach much importance to the occurrence of the cysts in the flea. But again there is the possibility that he was correct in his first assumption that the cysts in the flea were a stage in the extra-corporeal cycle of *H. jaculi*.

Miller (4) in the case of *H. perniciosum*, a haemogregarine attacking the leucocytes of the white rat, has described in the body of the mite *Lelaps echidninus*, a cycle of development exactly parallel in its main features to that outlined for *L. canis*.'

The formation of the large cysts containing numerous sporocysts and the packing of these in the body cavity exactly recall the conditions seen in ticks fed on dogs infected with *L. canis*. Not only so but in many of the details of the cycle described for *H. perniciosum* I recognise appearances seen and studied in the development of *L. canis*. Miller has gone so far in the case of *H. perniciosum* as to produce infection by feeding rats on mites whose bodies contained cysts.

In each of these cases then the same developmental cycle is clearly concerned, and the true or supposed cycles in *H. gerbilli*, *L. canis*, *H. jaculi*, and *H. perniciosum* all stand or fall together.

Proof that the developmental cycle described in the tick is really that of Leucocytozoon canis

Wenyon does not discuss the possibility that the forms described in the tick may be a natural parasite. His belief that they were actual developmental stages of *L. canis* was probably based, as were my own earlier observations, on finding them so regularly whenever ticks were fed on infected dogs, and on the fact that the various stages could be followed step by step from the encysted vermicules taken into the gut of the tick.

More than one error has, however, been made in the past in tracing what appeared to be consecutive steps in the development of parasites in external hosts, and when Miller published a complete cycle for *H. perniciosum*, which was clearly that with which I was dealing in the case of *L. canis* and *H. gerbilli*, it seemed to me that the most important question at issue then was whether by any possibility some commonly occurring type of arthropod protozoal parasite had not been concerned in each case. The following experiments which were undertaken some time ago, but have not yet been published, bear upon this aspect of the case. It was intended to repeat Miller's experimental infection of the host by feeding it upon infected specimens of the carrier, but the experiments were unfortunately cut short by other duties before this could be done.

1. Tick larvae fed on dogs with haemogregarines and on dogs free from infection.

26. 8. 07. Larvae fed on dog 22 (uninfected).
8 examined showed nothing.

26. 8. 07. Larvae fed on dog 20 (heavy infection).
9 examined showed nothing.

Other experiments with larvae fed on infected dogs not recorded were also negative.

2. Nymphs fed on dogs free from haemogregarines.

26. 8. 07. Nymphs fed on dog 22 (uninfected).
6 examined showed nothing.

3. 9. 07. Nymphs fed as larvae on dog 21 (uninfected) and as nymphs on dog 22 (uninfected).
8 examined very thoroughly. All negative.

4. 9. 07. 6 more of the same nymphs examined about 24 hours after dropping. All negative.

6. 9. 07. Nymphs fed as larvae on dog 21 (uninfected) and as nymphs on dog 29 (uninfected).
6 examined very thoroughly. All negative.

12. 9. 07. Nymphs fed as larvae on dog 21 (uninfected) and as nymphs on dog 33 (uninfected).
16 examined very thoroughly. All negative.

13. 9. 07. 12 more of the same nymphs 24 hours after dropping examined very carefully. All negative.

3. Nymphs fed on dogs whose blood contained haemogregarines.

3. 9. 07. Nymphs fed as larvae on dog 21 (uninfected) and as nymphs on dog 23 (fair number of haemogregarines).
8 examined. All showed vermicules but one. In three vermicules were fairly numerous, in four they were scanty.

3. 9. 07. Nymphs fed as larvae on dog 21 (uninfected) and as nymphs on dog 24 (numerous haemogregarines).
15 examined. All showed vermicules.

4. 9. 07. 6 more nymphs from dog 24 about 24 hours after dropping. All showed vermicules.

6. 9. 07. Nymphs fed as larvae on dog 21 (uninfected) and as nymphs on dog 24 (numerous haemogregarines). Same batch as fed with negative results on the same day on dog 29 (uninfected).
14 examined. All showed vermicules.

10. 9. 07. Nymphs fed as larvae on dog 21 (uninfected) and as nymphs on dog 40 (haemogregarines).
4 examined. All showed vermicules.

11. 9. 07. 7 more nymphs of same batch 24 hours after dropping. All showed developing vermicules.

4. Unfed adults fed as larvae and nymphs on uninfected dogs.

28. 8. 07. Adults fed as nymphs on dog 10 (uninfected).
12 dissected showed no cysts.

11. 9. 07. Adults fed as larvae on dog 12 (uninfected) and as nymphs on dog 21 (uninfected).
6 dissected showed no cysts.

5. Unfed adults fed as larvae on uninfected dogs and as nymphs on infected dogs.

16. 8. 07. Nymphs fed on dog 11 (numerous haemogregarines).
9 dissected on moulting. All showed numerous cysts approaching maturation.
Numerous other experiments not recorded showed that cysts in this stage were always to be obtained if the ticks had been fed as nymphs on heavily infected dogs.

6. Adults fed as nymphs on uninfected dogs and as adults on uninfected dogs.

24. 8. 07. Adults fed on dog 21 (uninfected).
3 dissected 24 days after dropping showed no cysts.

27. 8. 07. Adults fed as nymphs on dog 11 (uninfected) and as adults on a negative dog.
5 examined. None showed cysts.

5. 9. 07. Adults fed as nymphs on dog 12 (uninfected) and as adults on dog 33 (uninfected).
4 dissected on dropping showed neither vermicules nor cysts.

9. 9. 07. Adults fed on dog 38 (uninfected).
1 dissected showed no vermicules or cysts.

11. 9. 07. Adults fed as nymphs on dog 12 (uninfected) and as adults on dog 21 (uninfected).
8 examined for vermicules. All negative.
3 examined for cysts. All negative.

7. Adults fed as nymphs on uninfected dogs and as adults on infected dogs.

24. 8. 07. Adults fed on dog 23 (haemogregarines).
4 examined for vermicules and cysts showed numerous vermicules but no cysts.

5. 9. 07. Adults fed on dog 29 (fair number of haemogregarines) and allowed to commence oviposition.
3 examined. Oocysts present in all. No large cysts present.

24. 8. 07. Adults fed on dog 11 (numerous haemogregarines) examined 24 days after dropping.
Every specimen examined showed half-grown or nearly mature cysts. In many cases these were stained yellow and appeared as though no longer developing.
Numerous unrecorded experiments also showed that ticks fed on infected dogs showed vermicules or some of the various stages (depending upon the time when examined) described in my first paper or referred to in the first portion of the present one.

8. Adults fed as nymphs on infected dogs but as adults on uninfected dogs.

28. 8. 07. Adults fed as nymphs on dog 9 and as adults on dog 21 (uninfected).
6 dissected. All showed cysts but none vermicules.

1. 9. 07. Adults (source unknown but with free access during nymphal stage to numerous infected dogs) fed on dog 21 (uninfected).
3 dissected. Cysts were present in two. None showed vermicules.

2. 9. 07. 2 more adults from laboratory fed on dog 21. One showed cysts; neither showed vermicules.

These experiments, though more limited in number than was originally intended, are sufficient to prove that the developmental cycle dealt with was that of the haemogregarine.

The Development of *Piroplasma* in the Tick.

During the experiments with *Leucocytozoon canis* a number of ticks were examined which had been fed on dogs infected with *Piroplasma canis*. In such cases developmental forms as described by me in a previous publication were frequently seen.

The club-shaped forms are the most easily detected stage in the developmental cycle and can generally be found without difficulty about the fourth or fifth day in ticks which have been fed on heavily infected dogs. For their detection fresh preparations are made of the ovary, oviducts etc. and the field examined under a comparatively low power for the actively motile small leech-like immature club-shaped bodies or the more rigid and less active maturer forms.

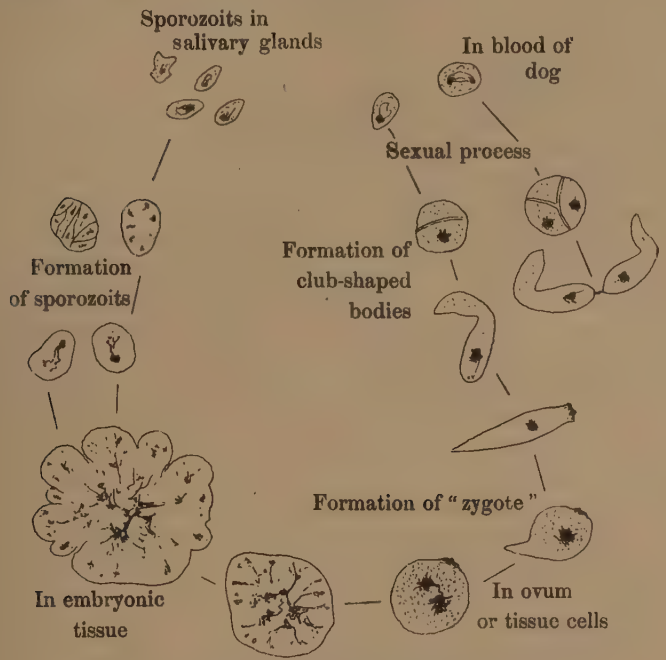

Diagram 2. Showing the developmental changes of *Piroplasma canis* in the tick.

Since my original description was published several authors have confirmed the view that the various appearances described by Prof. Koch and myself are stages in the development of *Piroplasma.* Marzinowski and Bielitzer's (8) work is the most detailed. They describe the development for *P. equi* as follows:

"One finds the star-shaped forms of Koch on the first, less frequently on the second day. They are, however, seldom to be found in stained preparations. Therefore it would be unwise to give them any great

diagnostic importance. (*Vide* also Nuttall and Graham-Smith (10).) From the second day onwards the parasites continue their progress and increase in size. Generally there are two types to be distinguished, the one, with dark blue coarsely granular protoplasm and a small nucleus, is generally of an oval or worm-like shape, the other has a somewhat smaller nucleus and lightly stained protoplasm.

On the second and third days one sees forms which point to the conjugation of two parasites of the same kind. One finds large masses of these pairs of forms in which the nucleus is hidden in the darkly staining protoplasm.

As a result of this conjugation new forms are built up which look like small worms. They are described by Koch and Christophers. In our own preparations we could observe the gradual changes. The worm-shaped forms are apparently 'ookinites' very lively, and are present in large quantities in the saliva of the ticks with which the eggs are wetted. Later on we find globular shaped forms of various sizes which are probably formed from the above mentioned worm-like bodies. In the large globular forms the chromatin has undergone division into small fragments. Besides these forms we succeeded in finding large numbers of granular globular bodies of various sizes. If these are various stages of development of *Piroplasma* as Koch takes it, or various cells of the tick it is at present difficult to decide. If, however, one may judge according to the analogy with other protozoa we may well accept the first suggestion."

In the egg these observers could find no parasites, though in the case of *Piroplasma canis* there is no doubt that forms occur embedded in the yolk. Koch also describes forms found in the egg.

Marzinowski and Bielitzer also describe and figure the small forms in the salivary glands. They say in conclusion:

"In this way we succeeded in observing the development of *Piroplasma* in the bodies of ticks and in the larva, our results coinciding completely with those of Koch and Christophers and being even partly supplemental to these."

However obscure the exact significance of the different stages may be, some idea of the general nature of the cycle of *Piroplasma* can be formed.

There is no doubt that certain parasites taken into the gut of the tick, after an enlargement as described by Marzinowski and Bielitzer, become club-shaped bodies and leave the gut. The formation of these club-shaped bodies I have followed very clearly. Marzinowski and

Bielitzer's fig. 18, Pl. VI, shows what is evidently a club-shaped body in process of formation as described on page 57 of my paper. The upper part of fig. 19 also shows the achromatic line to which I have referred, and the same line (indicating the formation of the tail of the club-shaped body) can be seen in several of the groups of parasites shown in fig. 17.

That the double bodies formed in the gut are not conjugation forms (as considered by Koch and Marzinowski and Bielitzer) is shown by the fact that these become not one but two vermicules. My explanation of this appearance is simply that a parasite on the point of division or already partly divided has taken on development. All the appearances seen favour this view.

I have also followed very clearly the change of the club-shaped bodies after they have embedded themselves in the yolk substance of the egg or, as happens when parasites are taken in during the nymphal stage, when they have penetrated the substance of tissue cells composing the embryonic-like mass, which models, as it were, the body of the adult tick within the skin of the nymph.

In both cases the persistence of the peculiar structure carried at the anterior end of the club-shaped body, and the fact that the sharp pointed tail can often be seen for some time projecting from the globular mass characteristic of the next stage, make any error of interpretation improbable. There follows then upon the club-shaped body, which suggests the ookinite, a stage of great growth within the substance of a cell. The arrangement of the chromatin in this stage clearly shows that an ultimate breaking up of the mass is intended. The appearances are not very dissimilar in fact to the earlier stages in the "zygote" of malaria as figured by Marchiafava and Bignami.

The appearances figured by Marzinowski and Bielitzer and referred to in the passage quoted as possibly being cells of the tick are clearly cells that have been invaded and almost converted into the products of division of the parasite. The figures recently given by Dschunkowski and Luhs (9) and others of *Theileria* multiplying in the tissue cells show exactly similar appearances.

The final accumulation of "sporozoites," or "spores" as they are termed by Marzinowski and Bielitzer, in the salivary acini is another undoubted stage. The figures given by Marzinowski and Bielitzer of these bodies showing stellate and oval forms closely resemble those figured in my own work. The only observation of these authors at variance with mine is the finding of club-shaped bodies in the saliva of

adult ticks in the act of oviposition suggesting penetration of the ova after these have been laid. In *Piroplasma canis* at any rate the penetration of the ova whilst still within the ovary can be demonstrated.

The outline of development indicated above enables one for the first time to form any conception of the reasons why in some cases transmission is hereditary and in others passed from stage to stage, and why in some cases hereditary transmission is effective in the larval stage whilst in other cases it occurs only at a later ecdysis.

Both in the haemogregarine and in *Piroplasma* there is some reason to believe that the parasite goes through one and the same cycle without reference to the particular stage of growth that the tick has reached. One can see how details in the life history of particular species of tick, or even different rates of development of the parasite, might bring about great differences in the transmission of infection without any great modification in the actual cycle of parasitic development.

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BEMERKUNG ZU DER ARBEIT OTTO V. HUFFMAN: "THE KURLOFF-BODY, A SPURIOUS PARASITE."

Von Dr V. SCHILLING-TORGAU,
Oberarzt, kdt. zum Institut f. Schiffs- u. Tropenkrankheiten, Hamburg.

Die unter diesem Titel in Vol. IV, No. 4 dieser Zeitschrift erschienene Arbeit V. Huffman's gibt mir Anlaß zu folgenden Bemerkungen, nicht weil ich ihre Ansichten nicht teilte, sondern weil sie geeignet ist, über meine Stellung zu der Frage der Kurloff-Körper einen unrichtigen Eindruck zu erwecken. Im ersten Absatz der Arbeit steht der Satz: "Therefore it is not with surprise that we find Ferrata, Patella, Goldhorn and Schilling and others believing these bodies to be protozoan in nature. Patella however is the only one who claims to have observed a flagellate develop from a Kurloff-body and of course this observation would put an end to all dispute if it could be verified." Die Arbeit wendet sich indessen nur gegen die Patella'schen Anschauungen, obgleich im Literaturverzeichnis meine Arbeit über die Aehnlichkeit dieser Einschlüsse mit Chlamydozoenkörpern zitiert ist. Die gegen Patella angeführten Beweisgründe decken sich im wesentlichen mit den meinigen (abgesehen von durchaus negativen Flagellatenuntersuchungen, deren Zugehörigkeit von vornherein sehr unwahrscheinlich war), *sind aber gerade im morphologischen Teile weit weniger eingehend und führen zu dem gleichen Ergebnis, daß die Kurloff-Körper sicher keine gewöhnlichen Protozoen sind.* Statt aber meine ihm bekannten Resultate zu bestätigen, erwähnt der Autor mich kurz unter den Anhängern der Protozoennatur der Kurloff-Körper. Mit der von mir betonten Aehnlichkeit mit Chlamydozoenkörpern ist keineswegs gesagt, daß sie wie diese noch ziemlich zweifelhaften Gebilde nun wirklich Erzeugnisse einer Infektion (höchstens mit ultravisiblen Erregern) sein müßten; ich habe ausdrücklich ausgesprochen, daß das, was von den Kurloff-Körpern dargestellt wird, eine *Reaktionsstruktur der Zelle selbst vielleicht ihres Kerns* auf eine *unbekannte* Ursache zu sein scheint.

Die Arbeit V. Huffman's bietet also wenig Neues und ist geeignet, über die Stellung der genannten Autoren (außer Patella) einen nicht richtigen Eindruck zu erwecken.

NOTES ON TICKS. II.

(1) NEW SPECIES (*AMBLYOMMA*, *HAEMAPHYSALIS*).
(2) *IXODES PUTUS*: DESCRIPTION OF THE HITHERTO UNKNOWN LARVAL STAGE.

By GEORGE H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

(With 9 Text-figures.)

The following descriptions relate to a new species of *Amblyomma* in all its stages, to two species of *Haemaphysalis* of which diagnoses of the males and females are given, and to the hitherto unknown larva of *Ixodes putus*:

Amblyomma darlingi (n. sp. ♂ ♀ o and larva), from Panama	1285–1287[1]
Haemaphysalis warburtoni n. sp. (♂ ♀), from China	1400
Haemaphysalis montgomeryi n. sp. (♂ ♀), from India	760–762
Ixodes putus (Pickard-Cambridge, 1878). Larva.	1307

Amblyomma darlingi n. sp.

Figs. 1–4.

Male (Fig. 1). *Body*, *L.* 1·8–2·6, *W.* 1·4–1·8 mm.[2], elongate, slightly flattened at the sides. *Scutum* with ornamentation as indicated by the

[1] These figures refer to numbers in our collection. The letters *L.* and *W.* in the diagnoses refer to the length and width of the body, exclusive of the capitulum. The letters *l.* and *w.* refer to the length and width of certain structures in the tick. The length of the capitulum is measured from the dorsal ridge to the tip of the hypostome.

[2] Five males gave the following measurements in mm.:

	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)
Body length	1·8	2·2	2·4	2·5	2·6
Body width	1·4	1·5	1·7	1·8	1·7
Capitulum length (dorsally)	0·6	0·6	0·9	0·8	0·8

light areas in Fig. 1, but subject to slight modification[1]; four ornate festoons separated by single inornate festoons one of which is the median. Scapulae moderately pointed. Lateral grooves extend forward either half-way or slightly more along body-length and continuous with a row of punctations which is continued forward. Cervical grooves from short deep oval pits fading away behind into shallow depressions.

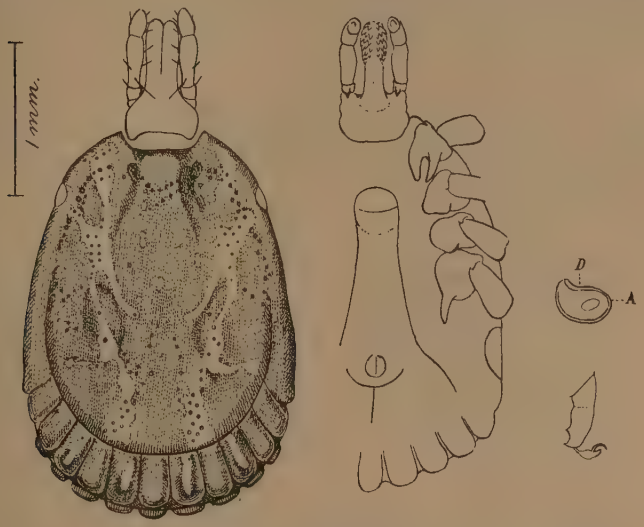

Fig. 1. *Amblyomma darlingi* ♂, dorsum and venter, spiracle and tarsus 4. (G. H. F. N. and E. W. del.)

Punctations few, medium, in groups, almost absent over the antero-median area. Two large elongate postero-lateral depressions. Eyes large, pale, oval, slightly protruding. Festoons deeply incised, longer than broad, *the trenchant edges of the ventral festoons protruding beyond the body and visible dorsally.* *Capitulum* (*l.* 0·6–0·9 mm.), base broader than long, slightly convex laterally, cornua rounded; base sub-quadrangular in ventral aspect. Palps normal, article 3 about twice as long as 2, article 1 with short ventrally protruding basal spine. Hypostome with corona, spatulate, dentition 3|3, 8 to 9 teeth on the outer file. *Venter*: genital orifice facing coxae II. Spiracle short comma-shaped. *Legs* slender; coxa I with two sub-parallel spurs of

[1] The modifications consist in the scapular patches being less pronounced and the absence of the small antero-median and lateral patches.

equal length or the outer slightly longer, coxae II–III with a slight protuberance; coxa IV with long spur pointing slightly inward. Tarsi ending bluntly, with two spurs. Pads almost attaining points of claws.

Female (Fig. 2). *Body* (unfed), *L.* 2·9, *W.* 2·1 mm., with marginal groove complete, festoons distinct. *Scutum* about as long as wide (*l.* 1·5–1·7, *w.* 1·6–1·9 mm.)[1], scapulae moderately pointed, emargination deep, lateral angles rounded, postero-lateral margins sinuous, posterior

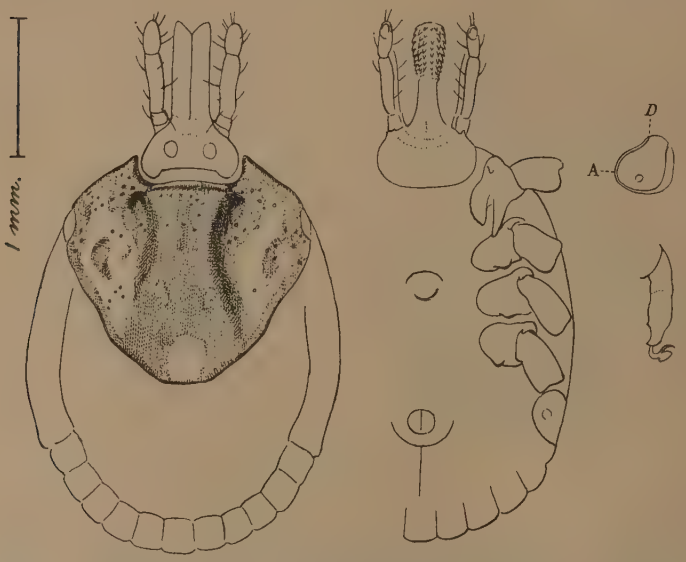

Fig. 2. *Amblyomma darlingi* ♀, dorsum and venter, spiracle and tarsus 4. (G. H. F. N. and E. W. del.)

angle truncate. Ornamentation consisting of two irregular patches starting at the scapulae, occupying the lateral fields, and a large postero-median patch tending to fuse, in some cases, with small patches in the middle field and anterior thereto. Eyes pale yellow, distinct,

[1] Four females gave the following measurements in mm. (no replete specimens):

	(*a*)	(*b*)	(*c*)	(*d*)
Scutum length	1·6	1·7	1·5	1·6
Scutum width	1·7	1·9	1·8	1·6
Capitulum length (dorsally)	0·9	1·1	—	1·0

flat, long-oval. Cervical grooves start as deep pits and fade away without attaining posterior border. Punctations few, medium, grouped laterally and anteriorly. *Capitulum* resembling that of ♂, *l.* 1 mm., porose areas small, deep, round, far apart. Palps: article 2 over twice as long as 3. Hypostome 3 | 3, 9–10 teeth per external file, very broad distally, with long narrow unarmed basal portion. *Venter*: vulva facing second intercoxal space (in half-gorged specimens it faces coxa II). Spiracle sub-triangular. *Legs* resemble those of ♂, but coxa I with external spur distinctly longer than the internal, coxae II–IV with blunt protuberance. Tarsi tapering slightly, 2-spurred.

Nymph (Fig. 3) resembles the ♀. *Body* (unfed), *L.* 1·1–1·2 mm.[1], when gorged, attaining *L.* 1·8 mm. *Scutum* broader than long (*l.* 0·6, *w.* 0·7 mm.), emarginate, with angular sides, postero-lateral border sinuous, cervical grooves starting as elongate reniform pits and not

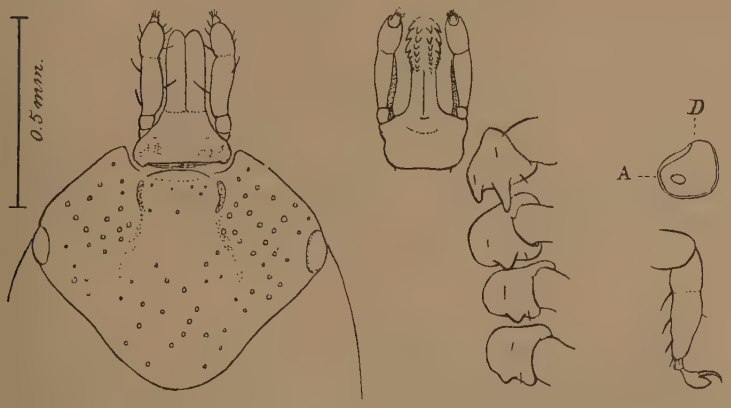

Fig. 3. *Amblyomma darlingi* ♀, parts of dorsum and venter, spiracle and tarsus 4. (G. H. F. N. del.)

attaining the posterior border. Eyes large, oval. Punctations fairly numerous, relatively coarse. *Capitulum*: *l.* 0·4 mm., base sub-triangular, external borders rounded, cornua slight, dorsal surface slightly corrugated; the base, viewed ventrally, sub-rectangular, with rounded angles and slightly indented external contour. Palps with article 2 about twice as

[1] The various measurements of nymphs and larvae were made on eight specimens chosen from amongst many because of the apparent differences they exhibited in point of size. The differences were, however, very slight or *nil*.

long as 3. Hypostome spatulate, 2|2, with 5–6 distinct teeth per external file. *Venter*: spiracle sub-triangular. *Legs*: coxae resembling those of ♀; tarsi tapering gradually, unarmed.

Larva (Fig. 4) resembles the ♂. *Body* (unfed), *L.* 0·5–0·6 mm.[1], when gorged attaining 1·1–1·2 mm. *Scutum* broader than long (*l.* 0·3, *w.* 0·4 mm.), faintly emarginate; cervical grooves parallel. *Capitulum*: *l.* 0·2 mm., base sub-triangular, lateral angles and antero-lateral

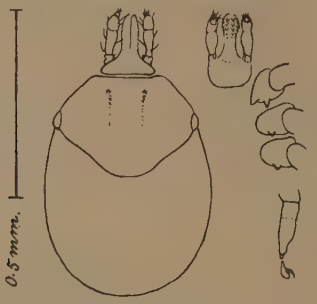

Fig. 4. *Amblyomma darlingi* L, dorsum, part of venter, tarsus 4. (G. H. F. N. del.)

borders slightly rounded; base, viewed ventrally, with rounded sides and flattened posterior border. Palps and hypostome resembling those of ♂. *Legs*: coxa I with two short divergent pointed spurs, the outer longer; coxae II–III with short blunt spur. Tarsi tapering gradually, unarmed.

Described from 5 ♂, 9 ♀, 40 ♂, and 23 larvae found on a deer (*Odocoleus* sp.), Corozal, Panama Canal Zone, 14. VIII. 1910, by A. H. Jennings, of Ancon, and 2 ♂, found on the nape and head of a turkey buzzard (*Catharista atratus*), Empire, Canal Zone, IV. 1911, by S. T. Darling, M.D., Chief of Laboratory, Isthmian Canal Commission, Ancon, Canal Zone. Named in honour of Dr Darling in view of his distinguished services to parasitology. The types, for which I am indebted to Dr Darling, are in Cambridge (N. 1285–1287).

Before establishing this new species, of which, fortunately, all the stages were secured, I referred some of the specimens to Professor L. G. Newmann (Toulouse) and to my colleague, Mr L. E. Robinson (Watford),

[1] The various measurements of nymphs and larvae were made on eight specimens chosen from amongst many because of the apparent differences they exhibited in point of size. The differences were, however, very slight or *nil*.

who is devoting special attention to the genus *Amblyomma* in conjunction with our monograph on Ticks.

The new species offers a superficial resemblance to *A. cajennense*, but differs especially in respect to the ornamentation in both sexes. The ♂ differs in being smaller and more elongate, the ♀ scutum is relatively broader and more triangular, not to mention other differences affecting the spiracles, coxae, tarsi, and cervical grooves in ♂ and ♀ and the festoons in the ♂.

Haemaphysalis warburtoni n. sp.

Figs. 5–6.

Male (Fig. 5). *Body, L.* 2·3–2·5 mm., *W.* 1·6–1·8 mm.[1], narrow in front, broadest on a line with the spiracles. *Scutum*: cervical grooves

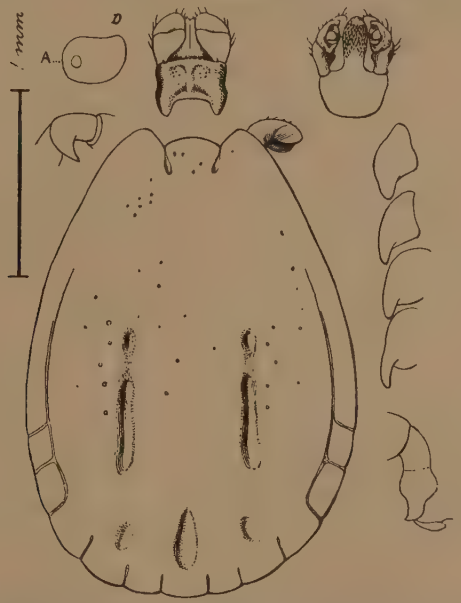

Fig. 5. *Haemaphysalis warburtoni* ♂, capitulum in dorsal and ventral aspects, scutum, spiracle, trochanter 1 seen from in front (and, attached to body, from above), coxae, tarsus 4. (G. H. F. N. and E. W. del.)

[1] Five males gave the following measurements in mm.:

	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)
Body length	2·3	2·4	2·4	2·4	2·5
Body width	1·6	1·6	1·7	1·7	1·8
Capitulum length (dorsally)	0·4	0·5	0·5	0·5	0·5

short, convergent, lateral grooves include two festoons and extend forward to two-thirds the body-length; posteriorly a median groove and two lateral depressions, two longitudinal grooves anterior to the latter extending forward to half the length; festoons short; punctations few, inconspicuous. *Capitulum*: *l.* 0·4–0·5 mm., base sub-rectangular, with concave dorsal ridge connecting stout, somewhat convergent cornua having rounded points; base bulges ventrally. Palps longer than broad, being broadest at the distal end of article 2, which is about a third longer than article 3. Hypostome broad, 4 | 4, with corona followed by 8 distinct teeth per file. *Venter*: genital orifice between coxae II; spiracle longer than broad, with recurved dorsal margin. *Legs* short, stout. Coxae I–IV each bearing a stout spur, longest on coxae IV and concave externally. Trochanter 1 with very large dorsal blade. *Tarsi remarkable*, the distal portion bulging dorsally and ventrally, tapering rapidly, bearing a spur. Pad half as long as the claws.

Female (Fig. 6). *Body* (unfed), *L.* 2·3 mm.[1], with marginal groove including the second festoon and almost attaining the scutum. *Scutum*: *l.* 1·2–1·6, *w.* 1·4–1·6 mm., cordiform, cervical grooves not attaining the posterior border, postero-lateral border almost straight, few inconspicuous punctations. *Capitulum*: *l.* 0·8–0·9 mm., base broader than long, with sides angular, the antero-lateral borders converging, dorsal ridge concave, wavy, connecting short stout cornua; porose areas long oval, converging anteriorly, far apart, in some specimens separated by a median depression; viewed ventrally, the base bulges markedly. *Palps atypical*, very long, with article 1 distinctly visible dorsally, article 2 about twice as long as 3, the palps being broadest at the juncture of articles 2–3. Hypostome broadly spatulate, 4 | 4 or 5 | 5, with emarginate corona, about 10 distinct teeth per file. *Venter*: vulva between coxae II when unfed, facing second intercoxal space when replete; spiracle as long as broad, somewhat angular, with slightly marked postero-dorsal angle. *Legs* resembling those of ♂, the spur on coxa IV less developed.

Described from 5 ♂ and 9 ♀ specimens, found on *Serow goat*, Wen-chwan-hsien, near Si-ho-hsien, China, and purchased, VII. 1911, from

[1] None of the specimens were fully gorged, the largest measured *L.* 6·1 mm. Four females gave the following measurements in mm.:

	(*a*)	(*b*)	(*c*)	(*d*)
Scutum length	1·2	1·5	1·5	1·6
Scutum width	1·6	1·6	1·6	1·4
Capitulum length (dorsally)	0·8	0·9	0·8	0·9

T. V. Sherrin, Taxidermist, Hampton. Types in Cambridge (N. 1400). Named in honour of my colleague, Mr Cecil Warburton.

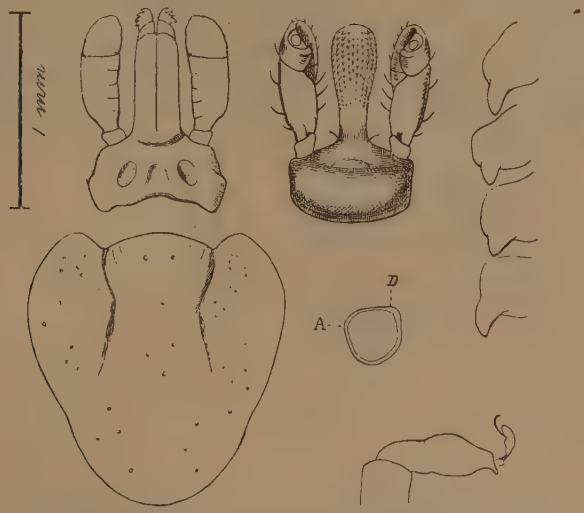

Fig. 6. *Haemaphysalis warburtoni* ♀, capitulum in dorsal and ventral aspects, scutum, coxae, spiracle and tarsus 4. (G. H. F. N. and E. W. del.)

Haemaphysalis montgomeryi n. sp.

Figs. 7–8.

Male (Fig. 7). *Body*, *L.* 1·9–2·5 mm., *W.* 1·3–1·6 mm.[1], long oval. *Scutum*: cervical grooves normal; lateral grooves including the first festoon and attaining half the body-length; festoons longer than broad. *Capitulum*: *l.* 0·4–0·5 mm., base with lateral borders almost straight, converging behind; cornua pointed, continuous with the crescentic dorsal ridge; ventral ridge sharp with trenchant lateral angles. Palps with article 2 protruding slightly, about a third longer than article 3; articles 2 and 3, viewed laterally (P. in Fig. 7), bear sharp protruding recurved spines ventrally. Hypostome 5 | 5 or 6 | 6, armed nearly to the

[1] Five males gave the following measurements in mm.:

	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)
Body length	1·9	2·0	2·3	2·3	2·5
Body width	1·3	1·4	1·4	1·4	1·6
Capitulum length (dorsally)	0·4	0·5	0·5	0·5	0·4

base with 12 distinct teeth per external file, besides finer denticles and a large corona. *Venter*: genital orifice between coxae II; spiracle large, with well-marked postero-lateral elongation. *Legs* relatively strong. Coxae I–IV with a long, pointed, retrograde spur, longest on coxa I; trochanters with pointed spurs. Tarsi short, tapering from near the pseudo-articulation, bearing a small distal spur, and, in some specimens, a slight median protuberance ventrally. Pads almost as long as claws.

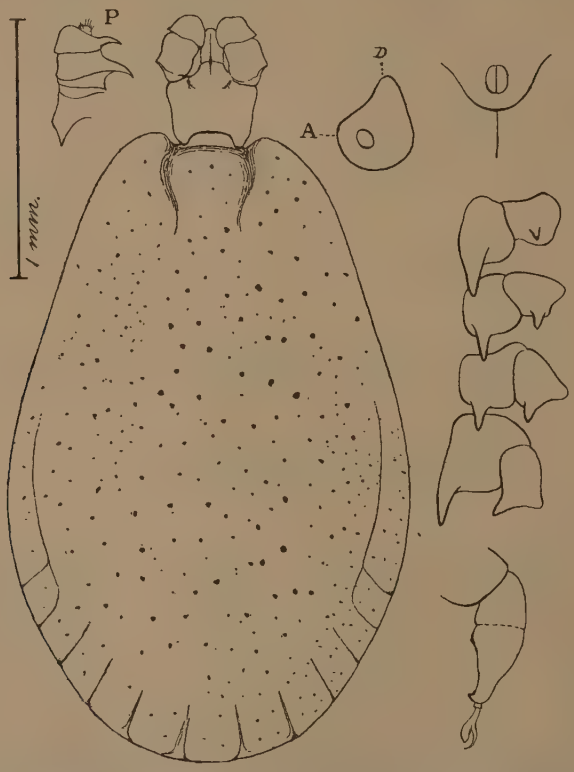

Fig. 7. *Haemaphysalis montgomeryi* ♂, dorsum, right palp (P) in profile, spiracle, anal grooves, coxae with trochanters, tarsus 4. (G. H. F. N. and F. M. H. del.)

Female (Fig. 8). *Scutum*: *l.* 0·9, *w.* 1·1 mm.[1], cordiform, with moderate lateral angles, broadly rounded behind. Cervical grooves

[1] All of the females were but partly gorged; they measured, roundly, *L.* 4 mm.

well-marked, extending slightly beyond half the length; punctations poorly marked, uniformly distributed. *Capitulum*: *l.* 0·5 mm., resembling that of ♂, but broader, shorter, with cornua less pronounced; porose areas oval, far apart, converging in front, placed anteriorly. *Venter*: vulva between coxae II; spiracle with dorsal and posterior

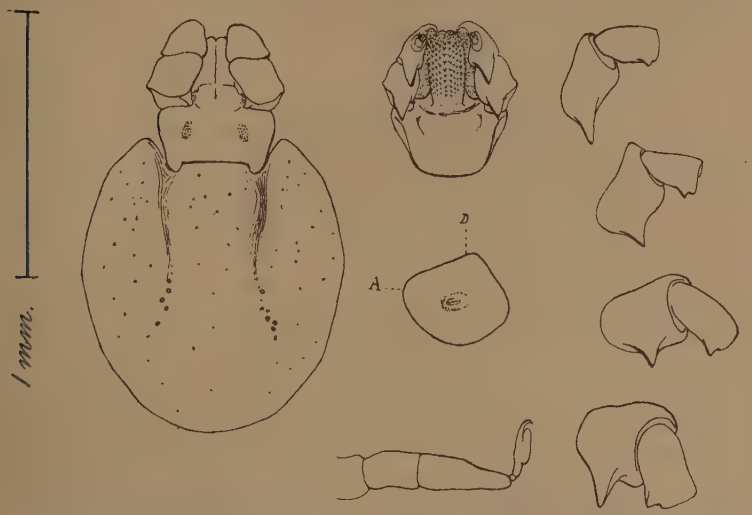

Fig. 8. *Haemaphysalis montgomeryi* ♀, capitulum and scutum, capitulum in ventral aspect, coxae and trochanters, spiracle and tarsus 4. (F. M. Howlett, del.)

margins flattened. *Legs* resembling those of ♂, but the spurs on coxae and trochanters less pronounced. Tarsi tapering gradually, unarmed.

Described from 9 ♂ and 2 ♀ specimens found on the ears of *ponies*, at Muktesar, United Provinces, India, 30. v. and 1–7. vi. 1905, also on a *bull's* ear at Bhulumaya; 3 ♂ found on a *dog*, Muktesar, 3. viii. 1905; 2 ♀ found on the ear of a *bull*, Berinag, U. P., India, 9. ix. 1905. All the specimens were collected by Dr R. E. Montgomery after whom the species is named. Types in Cambridge (N. 760, 761, 762).

Ixodes putus (Pickard-Cambridge, 1878).

Larva (Fig. 9). Closely resembles the nymph in all essential characters[1]. *Scutum*: *l.* 0·4 mm., narrow, approximating more to that of the ♀ in shape. Larvae, when gorged, attain 1·5 × 1·2 mm. *Capitulum*: *l.* 0·2 mm.

Egg: *l.* 0·6 mm., long, golden yellow, blunt oval.

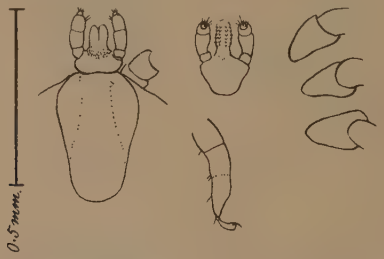

Fig. 9. *Ixodes putus* (Pickard-Cambridge, 1878) larva, part of dorsum, capitulum in ventral aspect, coxae, tarsus 3. (G. H. F. N. del.)

Described from numerous specimens found by me, 2. VII. 1911, with ♂, ♀ and ○ in nests on cliffs inhabited by marine birds, chiefly guillemots, Worm's Head, Gower, South Wales. No unfed specimens of larvae were found and the eggs failed to hatch out. Types in Cambridge (N. 1307 b.).

I shall refer to the biology of this species in a future note.

[1] For description of ♂, ♀ and ○, and figures thereof, see *Ticks*, Part II, pp. 256–261. In this connection I would note that an American reviewer of Part II has criticized our descriptions of the immature stages because of their brevity. It is however obvious that lengthy descriptions are unnecessary when *accurate figures* form an integral part of a description and the immature tick possesses the *essential characteristics* of more adult forms which have been adequately described elsewhere. The literature on ticks is already overburdened with long descriptions of such a vague character that they are of no use to the systematist.

NOTE ON *ROSSIELLA ROSSI* (NUTTALL, 1910) OCCURRING IN THE JACKAL IN BRITISH EAST AFRICA.

By GEORGE H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

In a paper published in April, 1910, I described and figured a new intracorpuscular parasite found in smears made from the spleen and liver of a jackal (*Canis adustus*), the animal having been shot by Mr W. F. Cooper in British East Africa. As I stated at the time, I hesitated about referring the parasite to the genus *Piroplasma*, and only did so provisionally because it offered a resemblance to two parasites found in the internal organs of one of my dogs which had "recovered" from *P. canis* infection. I, however, added: "Should future investigation prove that the parasite of the jackal differs from the types of *P. canis* encountered in 'salted dogs' I would propose to refer it to a new genus, *Rossiella* (*Rossia* being preoccupied), in view of its specific name."

The original description was based upon 98 parasites which were all that could be discovered after very prolonged search in the organ smears prepared by Mr Cooper. The scarcity of the parasites rather suggested the idea that the jackal might perhaps be regarded in the light of a "salted" animal, and consequently rendered it necessary to be cautious in interpreting the significance of the parasites.

Through the courtesy of Dr R. E. Montgomery, Veterinary Pathological Laboratory, Nairobi, British East Africa, I am now in a position to refer *P. rossi* definitely to a new genus. The parasite will consequently be called *Rossiella rossi*. Dr Montgomery has sent me preparations[1] comprising a blood-film and six smear preparations from

[1] The slides were prepared from a jackal which died 6. XII. 1911 at the Laboratory in Nairobi. Dr Montgomery writes that Mr Branwhite, an assistant at the Laboratory, had had some young jackals under his care for six weeks, the animals having been caught when about 3½ months old. Three of the jackal cubs died, in each case it would appear, suddenly. The preparations which were sent to me were derived from the third cub, and Dr Montgomery sent them to me in view of the parasites in the films corresponding with the description of *P. rossi*.

the liver, kidney and spleen of a jackal cub; three of the latter were stained on arrival in Cambridge. Whereas very few parasites could be detected in the organ smears, no less than 1·25 % of the corpuscles[1] in the blood-film were found to harbour parasites. We may conclude, because of the large number of parasites present in the blood-film, that the jackal was suffering from an acute attack of disease set up by the parasite.

The blood-film was carefully examined and six hundred parasitized corpuscles were classified in accordance with the types of parasites which they contained, the results being as follows:—

No. of R.B.C. examined	Percentage (roundly)	
302	50	Contained single uninucleate parasites, of which 42 % were small, 25 % medium-sized and 13 % large.
197	33	Contained two uninucleate parasites, of which 40 % were small, 53 % medium-sized and 7 % large.
72	12	Contained binucleate single parasites, *i.e.* dividing forms about to give rise to two (or more) parasites.
21	3·5	Contained four uninucleate parasites.
7	1	Contained two parasites each binucleate, *i.e.* in the act of giving rise to four parasites.
1	0·1	Contained three parasites, two being small and uninucleate; the other larger and binucleate, *i.e.* about to give rise to two parasites.
Total 600		

The parasite has already been adequately described in my previous paper, where the characters which serve to differentiate it from *P. canis* are also considered. The parasite cannot be confused with *P. gibsoni* Patton 1910 which occurs in the jackal in India.

There is little to add to the earlier description. Further observation has only confirmed what I have previously stated with regard to the parasite. I may, however, add the following remarks: In 19 out of the 600 parasites, small, single blue-staining spheres or ovoid bodies were seen lying apparently detached and to one side of the parasite; in three instances there were two such detached masses. These bodies may have been actually thrown off by the parasite, or possibly they were connected thereto by invisible strands of protoplasm; none of these bodies appeared to contain chromatin. In 15 out of the 600 parasites examined, a small intensely staining chromatin granule was observed

[1] 2000 corpuscles were counted.

lying alongside the nucleus, either connected to it or free and more or less distant from it. A similar structure has been described and figured in *Piroplasma*, but I have been unable to convince myself that the supposed structure is not due to a chance particle of stained substance occurring in this situation. Such particles are frequently present here and there in the best preparations, being, however, ignored by a casual observer unless they happen to occur in proximity to the nucleus.

Judging from the percentages of the different types of parasites present in the blood, i.e. taking them as indicators of the relative duration of the various phases in the development of the parasite, we find that the longest period is occupied in the growth of the single parasite. (They form 50 % of all the parasites enumerated.) The smallest forms measure 1·5 μ; they are the most numerous of the single parasites (40 %), and they probably grow rapidly at first (the medium-sized numbered 25 %) and very soon after attaining their full size, they subdivide—only 13 % of the single parasites being large. Having divided into two, the daughter cells must remain quiescent for some time for two rounded parasites are found in no less than 33 % of all the infected corpuscles[1]. Analyzing the pairs of parasites, we find that 40 % are small and 53 % are medium-sized, which appears to indicate that they grow *in situ*; the 7 % of large pairs suggests that these are the few which again subdivide, thus giving rise to the sets of four parasites which occurred in 3·5 % of all the infected corpuscles. That it takes an appreciable time for the daughter cells to divide after nuclear division has taken place is indicated by there being no less than 12 % of binucleate single parasites found amongst all the infected corpuscles. *The same line of reasoning will doubtless apply to the determination of the relative length of time occupied in the development of other blood parasites, and it should prove of value in the future*[2].

To summarize: *Rossiella* belongs to the family *Piroplasmidae* of França. It is an intracorpuscular non-pigment-forming parasite, occurring singly, in pairs and occasionally in fours within corpuscles, attaining a considerably larger size than *Piroplasma*, and usually possessing a rounded shape. The peculiar dividing forms and conjoined piriforms which are so characteristic of *Piroplasma* never occur. The

[1] This corresponds with what is observed in the case of conjoined piriforms in *Piroplasma*; they usually remain quiescent for a considerable time after being formed, that is why they yield such a high percentage of the total count.

[2] See also in this connection Nuttall and Strickland on p. 80 this volume.

nucleus is large; when at rest it is rounded. The parasite multiplies by direct division of the nucleus into two followed by division of the protoplasm, thus giving rise to two daughter individuals which, if sufficiently matured, may repeat the process of division within the same corpuscle. The chromatin structures in resting and dividing forms of *Rossiella* differ completely from those seen in *Piroplasma*. The protoplasm tends to take up the blue stain uniformly when the parasites are stained by modifications of the Romanowsky method, although some parasites show blue staining protoplasm condensed peripherally. Judging from stained specimens, the parasites are but slightly amoeboid. They may assume an irregular or elongated shape occasionally. In only five out of over 700 parasites which I have examined did these elongated forms possess a piriform shape which, I have no doubt, was transitory.

It will be a matter of considerable interest to study this parasite *in vivo*. Dr Montgomery has kindly sent me a gorged female *Haemaphysalis leachi*, of which three specimens were found upon the jackal whose blood harboured these parasites. I hope that the ticks are infected and that it will be possible to carry out further studies upon the parasites. At present we do not know, but can only suspect, that the parasite is conveyed by ticks.

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ON THE OCCURRENCE OF TWO SPECIES OF PARASITES IN EQUINE "PIROPLASMOSIS" OR "BILIARY FEVER."

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and C. STRICKLAND, M.A., B.C.,
Assistant to the Quick Professor.

(*From the Quick Laboratory, University of Cambridge.*)

(With Plate III, 8 Diagrams, 1 Text-figure and 5 Charts.)

"Piroplasmosis," or "biliary fever" in horses, has hitherto been regarded as a disease due to a distinctive parasite, *Piroplasma equi* Laveran. We propose to show that there are two specifically distinct parasites concerned in the production of biliary fever, and that consequently two distinct diseases have hitherto been confused under this name and under the name of piroplasmosis.

The parasite described as *Piroplasma equi* by Laveran (1901, p. 385) was studied by him in stained blood-films sent to him from South Africa by Theiler. Laveran referred the parasite to the genus *Piroplasma*, which, even at present, is made to include parasites which are distinctly different from this genus. As Nuttall and Graham-Smith have shown, parasites belonging to the genus *Piroplasma*, of which *P. bovis* is the type species, invariably multiply in a characteristic manner in that the peculiar piriform parasites develop through a process resembling budding from a large, rounded, or slightly amoeboid parasite the whole of whose protoplasm flows into the "buds" and gives rise as a rule to two piriform parasites without a residual body being left. As species of true *Piroplasma* we have hitherto

recognised *P. bovis*, *P. canis* and *P. pitheci*[1]. These species occur inside the corpuscles grouped in large numbers in pairs of piriform parasites connected at their pointed extremities. In *P. canis* a considerable number of corpuscles contain two pairs of piriform parasites, and at times higher multiples may be encountered.

Laveran's description and figures show that his *Piroplasma equi* does not possess the characteristic mode of multiplication and form of a true *Piroplasma*. The mode of multiplication as described by Laveran from stained specimens alone is moreover inaccurate. Convinced of the difference between Laveran's parasite and *Piroplasma*, França (1909) has more recently placed the parasite in a new genus, named *Nuttallia*. The parasites belonging to this genus do not multiply according to the method described for *Piroplasma*; they do not occur as pairs or multiples of pairs of piriform parasites inside of corpuscles, and they form distinctive "*cross forms*" which Bowhill (1905) and França (1909) regard as multiplication forms. According to França, then, *Piroplasma equi* Laveran 1901 = *Nuttallia equi* (Laveran) França 1909.

That two types of parasites occur in equine piroplasmosis was pointed out by Robert Koch (1905, p. 1867) who believed that they might cause distinct diseases, and here the matter rested until December 1910 when we published a short preliminary note upon the results of our investigations, which clearly show that two distinct species of parasites occur in horses suffering from biliary fever, and consequently that two distinct diseases are included under this name.

Through the courtesy of Sir John McFadyean, of the Royal Veterinary College, London, we obtained a strain of *N. equi* derived from South Africa and maintained by passage through several horses in London. The strain was sent to Cambridge in citrated blood taken from a horse which had been inoculated with a positive result two years before.

A detailed study of this parasite, both in fresh blood preparations and in stained films, quickly convinced us that França's view is correct, namely, that *P. equi* Laveran is not a *Piroplasma*, and we of necessity concur in referring the parasite to the genus he has done one of us the honour of naming *Nuttallia*. Our studies upon the living parasite, moreover, confirm the view that the cross forms are multiplication forms as the sequel will show.

So as to avoid confusion we shall first of all describe our observations on *N. equi* (Laveran 1901) and afterwards deal with the true

[1] Nuttall and Graham-Smith (VI. 1908), p. 134 etc.

Piroplasma (*P. caballi* Nuttall 1910) which also occurs in horses suffering from "biliary fever." As one of our *P. caballi*-infected horses was inoculated with *N. equi* after it had recovered from the clinical symptoms produced by the first-named parasite, it will make matters clearer to show the course of the experiments in the form appended:—

Nuttallia equi

Horse I. Inoculated 27. XI. 1909 with blood obtained from Sir John McFadyean. Parasites appeared 8th day. Horse died 11th day.

Horse II. Inoculated 8. XII. 1909 with blood from *N. equi* Horse I. Parasites appeared 7th and disappeared 22nd day. Horse recovered, and is still alive, 27 February, 1912.

Horse III. Inoculated 2. VIII. 1910 with blood from *N. equi* Horse II. Parasites appeared 10th day. Horse died 20th day.

Piroplasma caballi

Horse I. Inoculated 4. VI. 1910 with blood obtained from Dr E. J. Marzinowsky. Parasites appeared 15th day. Horse died 19th day: 22. VI. 1910.

Horse II. Inoculated 22. VI. 1910 from *P. caballi* Horse I. Parasites appeared 8th day. Horse recovered. (Subsequently inoculated with *N. equi*[1].)

Horse III. Inoculated 18. VII. 1910. Parasites appeared 9th day. Horse died 19th day.

[1] *N. equi* Horse III is the same animal as *P. caballi* Horse II. The *P. caballi* strain was carried on from Horse II to III before *P. caballi* Horse II was inoculated with *N. equi*.

I. NUTTALLIA EQUI (Laveran).

(A) *The living parasites.*

Although we spent roundly 200 working hours in the continuous study of the living parasite in fresh blood-films maintained at body-temperature, the results of our observations can be summarized briefly. The following summary is based on the continuous observation, coupled with graphic records[1], carried out on

	Average duration of observation on each parasite in minutes
41 small and medium-sized parasites	79
43 medium and large-sized parasites	106
8 pairs of small and medium-sized parasites	83
38 dividing and cross-forms	95

The number of infected corpuscles as compared to uninfected ones was small. Thus, in Horse I the number of infected corpuscles did not

[1] The accompanying diagrams are selected from a large series of graphic records.

exceed 5·4 %; in Horse III they attained 13·2 %. In films obtained from another horse, and which were kindly lent us by Sir John McFadyean, there were 6·6 % of infected corpuscles. In the case of Horse II the parasites appeared in the blood in such small numbers that there was no adequate opportunity of studying them.

Diagram I. N. equi. Illustrating the movements and alterations of form of a small parasite which was under observation from 14 minutes until 112 minutes after the blood was drawn. The corpuscle finally became crenated. The parasite appeared alternately oval, piriform and amoeboid, etc. protruding at one time two pseudopodia. The parasite seemed to grow in size. (Horse I, 6. XII. 1909, G.H.F.N. del.)

(1) *Small parasites* (Diagram I) measuring about 1 to 1·4 μ in size are frequently observed to be amoeboid. They move about within the corpuscle in an irregular manner whilst altering their shape, being at different times either oval, rounded, or piriform; or they may protrude a slight bud-like process. Some of this alteration of form is attributable to the rotation of the parasites within the corpuscle. The parasite may occasionally protrude a long filiform process, more rarely do they protrude two bud-like or filiform processes. These processes may be protruded or retracted slowly or within a couple of minutes. In some cases a rounded parasite was seen to protrude a long straight or curved filiform process extending two-thirds across the width of a corpuscle, the parasite undergoing but very little alteration in form for upwards of three hours afterwards. The thread-like processes at times showed a terminal swelling. When the parasites assumed a piriform shape it was only transitorily. The escape of small single parasites from corpuscles was never witnessed, and small parasites were never seen to divide. In some cases we believe that the parasites increased slightly in size during the time they were under observation.

Diagram II. N. equi. Illustrating the behaviour of two small parasites within a corpuscle during the period 24 to 61 minutes after the blood was drawn. The parasites appeared alternately rounded, piriform and of irregular form. (G. H. F. N. del.)

When two small or medium-sized parasites occur within a corpuscle (Diagram II) they behave in a manner similar to that of the single parasites above described.

(2) *Single medium-sized and large parasites* (Diagrams III–IV) behave very much as do the small ones. They change their shape more or less rapidly from round to oval or piriform, or they protrude one or two long processes which may or may not possess terminal swellings. We rarely observed the protrusion of three pseudopodia by amoeboid parasites.

Diagram III. N. equi. Illustrating the behaviour of a large parasite within a corpuscle during the period 17 to 176 minutes after the blood was drawn. The parasite appeared alternately rounded, amoeboid and piriform, and finally became rounded when all activity ceased. (Horse I, 6. XII. 1909. G. H. F. N. del.)

Diagram IV. N. equi. Illustrating the behaviour of a large parasite within a corpuscle during the period 19 to 237 minutes after the blood was drawn. The parasite appeared oval at first, then protruded a pseudopodium which caused it at times to appear piriform; then it protruded two pseudopodia so that it somewhat resembled a dividing form of *Piroplasma*. Finally, it became rounded and ceased to exhibit movements. (G. H. F. N. del.)

(3) *Very large single parasites*, of which a certain number are to be found, may show very little change of form, although they be watched for hours. They may remain rounded for long periods of time or assume an elongate[1] or plump piriform shape which is usually more or less transitory.

Diagram V. N. equi. Illustrating more especially the mode of multiplication of *N. equi* as observed in several infected corpuscles.

Fig. A. A single parasite observed through successive stages to the formation of four free oat-shaped or piriform parasites (Horse III, 13. VIII. 10, blood at 41° C., G. H. F. N. del.).

Fig. B. A "cross-form" which gradually gives rise to five parasites which move about inside the corpuscle (Horse I, 7. XII. 09, 39° C., H. B. F. del.).

Fig. C. The later stages in the development of a single parasite into six parasites (Horse I, 6. XII. 09, 38° C., C. S. del.).

Fig. D. A "cross-form," of which the component elements separate and swim about actively in the corpuscle prior to escaping without injuring the corpuscle (Horse III, 13. VIII. 10, 35° C., G. H. F. N. del.).

Fig. E. Whereas in the preceding instances the mother cell divides directly into 4–5–6 daughter cells of equal size, we have in this case what would appear to represent a budding-off process of small daughter cells which are liberated successively (Horse III, 12. VIII. 10, 37° C., G. H. F. N. del.). In Fig. F apparently a similar process is taking place (Horse II, 17. XII. 09, 37° C., H. B. F. del.).

[1] Such parasites may extend across the whole width of the corpuscles. The latter, in the horse, average 5·6 μ in diameter.

(4) *Dividing forms*: In a number of cases stages of development of a single parasite into four were observed, and the four young parasites were seen to separate. In one case (Diagram V, A) a previously rounded parasite was seen to give rise to four parasites after developing into a "cross-form." *In most cases* the cross-forms break up into four distinct parasites which wander away from each other. In three cases, what appeared to be an ordinary cross-form, gave rise to five parasites (Diagram V, B), and in one case six parasites resulted from the process of division (Diagram V, C). Young parasites, soon after they separate, may be very active and move about within the corpuscle (Diagram V, D).

In a few cases multiplication occurred by a budding process (Diagram V, E and F). Appearances may well be deceptive in such cases, for at times, when the infected corpuscle ruptures, the main mass of the parasite's protoplasm is seen to be connected to the supposed daughter individuals by delicate strands of protoplasm, which, owing to the haemoglobin within the corpuscle, had previously been invisible.

(5) *The escape of young parasites from corpuscles* was observed in a number of instances to occur shortly after they had become separated, the young parasites having arisen from a cross-form. The parasites did not rupture the corpuscle in the act of escaping. Immediately after their escape, singly or all together, the parasites appeared ovoid or piriform; they at times swam about actively for a few moments, after which they usually vanished, the conditions *in vitro* being doubtless unfavourable to their continued existence. Although we are convinced that the young parasites under natural conditions immediately re-enter fresh corpuscles after the manner of *Piroplasma*, we did not actually succeed in observing their entry into another corpuscle. The minute size of the parasites left us in doubt as to whether they lay upon or within the fresh corpuscle. On one occasion we saw a medium-sized parasite escape and degenerate outside the corpuscle which it left uninjured. It was noted several times in observing the microscopic field that the first corpuscles whose contained parasites escaped were those that harboured four young parasites. Some of these corpuscles vanished whilst the contained parasites appeared to be motionless, in other cases the corpuscles burst suddenly and the parasites were all ejected into the plasma.

(B) *The stained parasites.*

Having studied the living parasite, we were in a better position than previous observers to understand the significance of the forms which are encountered in stained films. Certain forms, however, still require investigation. Reference to the protocols relating to the horses upon which we experimented (see Appendix, *N. equi* Horses I–III, pp. 85–89 and charts) shows the results of the examination of their blood during the course of the disease induced by this parasite.

The parasites stain in a similar manner to *Piroplasma*. The hyaline cytoplasm takes on a blue colour with Giemsa's stain and the nuclear structures are stained deep carmine. The blue staining cytoplasm may appear more or less vacuolated or condensed peripherally. Definite nuclear changes precede the division of the cytoplasm; these changes are considered on p. 75 (Diagram VII) and p. 84 in the description of Plate III.

Time when the parasites appear in the blood. Percentage of infected corpuscles.

Horse I. The parasites appeared in the blood on the morning of the eighth day after inoculation, roundly 0·6 % of the corpuscles being found infected in the afternoon. They gradually increased in numbers up to the afternoon of the tenth day, when 5·4 % of the corpuscles were infected; and on the eleventh day, when the horse died, they had decreased slightly in numbers.

Horse II. The parasites appeared on the seventh day after inoculation. They gradually increased in numbers so that roundly 1 % of the corpuscles were found infected on the tenth and eleventh days. Their number then fell rapidly so that only isolated parasites could be detected up to and including the 21st day, after which they disappeared and the horse recovered.

Horse III. The parasites appeared on the tenth day after inoculation, 0·2 % of the corpuscles being found infected. They increased in numbers up to the twelfth day, when 11·6 % of the corpuscles contained them. They persisted in fair but somewhat variable numbers (5 to 13 % of the corpuscles being infected) from the 13th to the 20th day, when the horse died.

The number of infected corpuscles enumerated in these horses attained at most 5·4 %, 0·9 % and 13·2 %, respectively. The parasites

in Horses I, II and III appeared with the onset of fever; their number rose and fell with the body-temperature as will be seen by reference to the charts given in the Appendix.

The types of parasites encountered in the blood.

Owing to the changes of form observed by us in living parasites we have not attached much importance to the different shapes seen in parasites which have been fixed and stained. Naturally, they may assume a great variety of forms in death, these forms corresponding to what has been described in the living parasite. Enumerations of parasites according to their relative size lead us to no definite conclusions with regard to the relative prevalence of small, medium or large parasites at any stage of the disease. In Horse I the medium and large-sized parasites predominated over the small until the day of death, when there was an increase in small parasites; in Horse II the large parasites predominated more or less considerably over the small forms in 15 out of 19 counts, and there was no increase of small forms toward death[1]. About 90 % of the infected corpuscles contained single parasites of all shapes and sizes, usually 2–5 % contained 2 to 4 parasites and 1–5 % contained dividing or cross-forms[2]. Whereas in Horse III only few free parasites were usually encountered (0·2 to 6 %), in Horse I as many as 26 % of free parasites were encountered on the day of death, thus indicating that the corpuscles were rapidly breaking up and liberating the contained parasites[1].

These enumerations of the different types of parasites encountered in the blood offer a marked contrast to what is observable in *Piroplasma* (see p. 81).

The accompanying Diagram VI represents what appears to be the usual mode of multiplication of *N. equi* in the circulating blood, judging from the observations made in the first instance upon living parasites, and in the second upon stained specimens. The reader is referred to Plate III and its accompanying description for further details.

[1] In this connection it should be noted that Horse III had haemoglobinuria and jaundice but died without marked lesions, whereas the contrary was the case in Horse I. The latter's spleen weighed 7½ lbs., the liver 12¼ lbs., and extensive abdominal haemorrhage was detected at autopsy.

[2] In films from South Africa and Italy kindly lent us by Sir John McFadyean and by Professor A. Negri (Pavia) respectively, the dividing or cross-forms represented 7·2 % and 4·4 % of the *N. equi* present in blood-films.

N. equi multiplies slowly and in the following manner:

(1) The minute piriform or oat-shaped parasite enters a fresh corpuscle and (Diagram VI, 2, 3, 4, 5) grows in size, being slightly amoeboid, with a general tendency to resume a pear-shape. Definitely amoeboid movements (6) are, however, only to be seen distinctly when the parasite has attained a certain size. Judging from the form of the chromatin masses, stages 7, 8, 9, 10 follow next. The rest of the cycle has been continuously observed in the living parasite: the formation and breaking-up of the cross-form, the scattering of the daughter cells within the corpuscle, and their escape from the corpuscle,

Diagram VI. Illustrating the usual cycle of development of *N. equi* in the circulating blood. (Consult text pp. 74–76.)

which usually does not appear to be injured in the process. As previously stated, we find that corpuscles containing four parasites are liable to "vanish" earlier than do others.

(C) *Distribution of the parasites in the body.*

Immediately after the death of the horses (Nos. I and III) smears were prepared from the internal organs and stained, and the percentage of infected corpuscles and of different types of parasites enumerated. The counts, which we need not give here, revealed no more than did those made from the peripheral blood during life. The percentage of infected corpuscles appeared to be fairly uniform in all the organs examined, viz. spleen, liver, kidney, suprarenal glands, lung, heart-muscle, brain, and blood taken from the jugular vein and heart, etc., gave similar counts to those obtained with peripheral blood. When the parasites were classified according to types, the predominant forms were small, ranging from 50 to 80 % of the total number of parasites observed in the organ smears. Judging from the percentage of extracorpuscular forms, the breaking down of the infected corpuscles in Horse I took place sooner than in Horse III on the approach of death; the blood from the various organs of Horse III contained from 14 to 22 % of the parasites free in the plasma, whereas at no time were more than 5 % of free parasites detected in the peripheral blood of this horse whilst the animal was alive.

Observations on the blood.

In the case of *N. equi* Horse III (refer to protocol and chart in the Appendix, p. 88), which had been previously infected with *P. caballi*, blood counts showed a fall in the number of red blood corpuscles as the disease progressed. When the parasites appeared there were roundly nine million red blood corpuscles present per c. mm.; on the 12th day they had fallen roundly to seven million, and the fall continued so that on the 19th day only three million were present. The haemoglobin showed an almost corresponding fall. The leucocytes were markedly increased on the two days preceding the day when the horse died. Differential leucocyte counts were made on only four days; they showed a marked decrease in the number of eosinophiles. Nucleated red blood corpuscles were found on the 17th and 19th day. The blood was not studied in Horses I and II. In Horse III there were haemoglobinuria and jaundice.

II. PIROPLASMA (BABESIA) CABALLI.

(A) *The living parasites.*

Although the scarcity of parasites in the blood of the infected horses prevented our making as complete a study of their development *in vivo* as we should have wished, we nevertheless convinced ourselves that *P. caballi* (Diagram VII) develops in a manner similar to *P. bovis* and *P. canis*. Small and medium-sized parasites are usually oval or rounded in shape; they may, however, assume a somewhat piriform shape momentarily. When such parasites escape from corpuscles they degenerate and die. When large single piriform parasites occur in corpuscles (Diagram VII, D) they move about slowly in the corpuscle

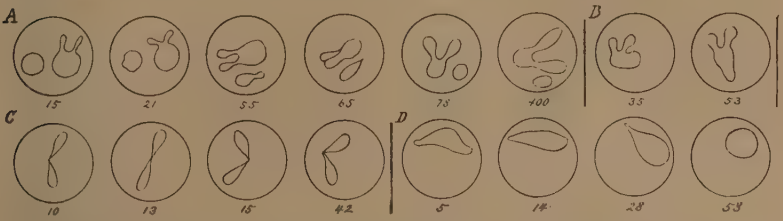

Diagram VII. P. caballi. Illustrating living parasites observed in fresh blood-films at blood temperature. (Horse I, VI. 1910.)

Fig. A. Two parasites, the larger, observed 15 minutes after the blood was drawn, almost developed into two piriform parasites at the end of 400 minutes. The smaller parasite underwent changes of form from round to piriform and oval. (G. H. F. N. del.)

Fig. B. A single parasite: early stages of multiplication observed 35–53 minutes after the blood was drawn. (C. S. del.)

Fig. C. Two conjoined piriform parasites showing changes of position within the corpuscle during 10–42 minutes. (G. H. F. N. del.)

Fig. D. Large piriform parasite slowly altering its shape and finally becoming rounded. The observation lasted 58 minutes. (C. S. del.)

whilst retaining their piriform shape; as in *P. bovis*, some large piriforms may extend almost across the whole width of the corpuscle. When moving about, such piriforms may occasionally appear rounded owing to their movements within the corpuscle being directed towards or away from the observer. Piriform parasites occurring in pairs were seen, as in *P. canis*, to escape from a corpuscle which afterwards vanished. Typical multiplication forms (Diagram VII, A and B) were seen as in *P. canis* and *P. bovis*, the "buds" persisting and leading almost to the complete formation of paired piriforms. The process of

multiplication, like that of *P. bovis*, is slower than in *P. canis*, but otherwise it is similar, and consequently requires no further description. Very large transitorily piriform parasites are frequently observed as in *P. bovis*.

Diagram VIII. P. caballi. Illustrating appearances seen in *Giemsa-stained parasites*. Selected figures from a large series of coloured drawings by C. S. made from films derived from Horses I and III and from films supplied by Dr Marzinowsky. The figures show the mode of multiplication. The blue-stained protoplasm is indicated by light stippling, the deeply-staining chromatin by black, and the pink or faintly-staining chromatin by close stippling. The contours of the corpuscles are schematized.

Fig. 1. Single piriform parasite which has recently entered a corpuscle. A deeply-staining chromatin mass lies to one side of the pink-staining body and upon or within a small spherical mass of faintly staining chromatin.

Figs. 2–4. Single parasites which have developed in size. Such parasites may be rounded but usually assume a piriform shape whilst slightly altering their form. The chromatin mass increased in size and frequently becomes elongated.

Fig. 5. Single parasite showing a large irregular faintly pink-staining chromatin mass.

Fig. 6. Parasite with dark-staining chromatin mass prolonged into a thread which terminates into a bead-like mass.

Figs. 7–9. The chromatin protruded in a fork-like manner into the buds of blue-staining protoplasm. (Figs. 7–18 as in *P. canis*, *P. bovis* and *P. pitheci*.)

Figs. 10–15. Showing more advanced stages of multiplication, a large mass of chromatin being seen at the point of bifurcation at the base of the buds.

Fig. 16. Showing the separation of the chromatin masses in the daughter cells which remain connected at their pointed ends.

Fig. 17. Newly-formed daughter cells still containing drawn-out masses of chromatin.

Fig. 18. Mature pair of piriform parasites, and which are ready to separate and escape from the corpuscle.

(B) *The stained parasites.*

A study of the stained parasites (Diagram VIII) shows that they are very closely allied to *P. bovis*, the large parasites being less actively amoeboid than in *P. canis*, and tending to assume a piriform shape. The chromatin structures are identical with those of *P. bovis*. The reader is referred to the Appendix for particulars relating to the course of the disease in the horses upon which we experimented (*P. caballi* Horses I, II and III).

Time when the parasites appeared in the blood. Percentage of infected corpuscles, etc.

Horse I. The parasites appeared in the blood on the 15th day after inoculation; they had increased slightly in numbers by the 18th day. The horse died on the 19th day from pulmonary haemorrhage. The disease (piroplasmosis), being but in the initial stage, the organs showed no distinct lesions.

Horse II. A few parasites appeared on the eighth day after inoculation and then disappeared, the animal recovering.

Horse III. The parasites appeared on the tenth day after inoculation; by the 13th day only 6 per 30,000 corpuscles were found infected, after which there was an intermittent increase of parasites; but even on the 19th day, when the horse died, only 0·5 % of the corpuscles were found to contain parasites.

At autopsy, this horse showed marked jaundice of the mucous membranes and subcutaneous tissues; the lungs were congested, the kidneys oedematous; the spleen was much enlarged, semi-fluid and very friable; it weighed 15½ lbs.; the liver was enlarged.

A rise in body-temperature preceded the appearance of the parasites in the blood of the three horses.

The types of parasites observed.

Consideration of the types of parasites encountered in the peripheral blood during life shows that the predominant forms are round (O) or double piriforms (PP). In Horses I and II only (O) and (PP) were found in the blood, but they were very scarce. Other typical forms were however encountered in the blood of Horse III whilst it lived, and in this case counts of the different types were rendered

possible, although the number of infected corpuscles only attained 0·5 %.

When we average the results of the counts of types of parasites made during four days on one of our horses suffering from *P. caballi* infection, we find the following percentages of the different types present in the blood, free parasites being omitted:

(P)	(O)	(D)	(PP)[1]
6·5	60·8	4·8	27·6

The average of three counts on blood-films prepared by Dr E. J. Marzinowsky, in Moscow, gave corresponding figures:

(P) (O)	(D)	(PP)
65	5	30

These percentages indicate roughly the relative duration of the various stages in the cycle of development of the parasite from the single piriform parasite on to the formation of (PP); the stages which last longest are the (O) and the (PP), that is why they yield a higher percentage in the counts. The duration of the (PP) stage is usually shorter than the (P) + (O) stage; thus in *P. caballi* the average of seven counts yield 67 % (P) + (O) to 32 % (D) + (PP). The (P) stage is usually short for the parasite soon becomes rounded or amoeboid after entering a fresh corpuscle. The proportion of free parasites is usually small except when the blood corpuscles are breaking up rapidly at the end of the disease, because the free Ps almost immediately re-enter fresh red blood corpuscles. When (PP)s are classified according as the parasites are conjoined at their pointed ends or separate in the corpuscle, we find that for every 100 conjoined pairs there occur only about ten separated piriforms—in other words the parasites usually escape soon after they cease to be conjoined.

[1] The signs indicate corpuscles containing
(P) a single piriform parasite.
(O) a single rounded parasite.
(D) a dividing parasite.
(PP) two piriform parasites.

(C) *Comparison of types of parasites encountered in different species of Piroplasma.*

A comparison of the different species of *Piroplasma* in respect to the predominant types which are encountered in the peripheral blood, only *P. pitheci* being omitted, shows that they all agree fairly well:

Species of Piroplasma	% of types encountered (P)	(O)	(D)	(PP)	(OO)	(P_4)[1]	Reference	No. of counts averaged (each representing 250 to 500 parasites)
Caballi	6·5	60·8	4·8	27·6	—	—	N. & S.	4
Caballi	65		5	30	—	—	N. & S.	3
Bovis	55		11	34	—	—	Nuttall & Graham-Smith (1908, p. 139)	2
Bovis	54		6	40	—	—	Nuttall & Hadwen (1909, p. 247)	7
Bovis	55		6	39	—	—	Nuttall & Hadwen (1909, p. 244)	7
Canis	5·0	45·0	0·7	43	1	4	Nuttall (1910, p. 423)	7
Canis	0·6	54·0	3	41	0·6	0·7	Nuttall & Hadwen (1909, p. 171)	7

If we group the numbers in two columns according as the infected corpuscles contain a single parasite or two or more parasites we obtain the following:

	Corpuscles containing: Single parasites	Two or more parasites	No. of counts averaged
P. caballi	71	29	7
P. bovis	61	39	7
P. canis	54	42	14

In the above table the forms (P), (O) and (D) are reckoned as one parasite. The results of the enumerations in the case of *Nuttallia equi* are totally different, even when we include the dividing or cross-forms in the second column; thus we obtain:

	Corpuscles containing: Single parasites	Two or more parasites and (D)s	No. of counts averaged
N. equi	96	4	7
N. equi	95	5	19

These counts, therefore, indicate, apart from the differences in morphology, that *Piroplasma* and *Nuttallia* are distinct parasites.

[1] The signs (OO) and (P_4) indicate corpuscles containing respectively two rounded and four or more piriform parasites.

Observations on the blood.

In the case of *P. caballi* Horse III (refer to protocol and chart in the Appendix, p. 92) there was a relatively slight decrease in the number of red blood corpuscles (from about 6½ to about 5 millions) and a slight fall in the amount of haemoglobin. The leucocytes showed a decided decrease as the disease advanced, accompanied by a disappearance of the eosinophiles and an increase in the neutrophiles.

III. Immunity test with regard to N. equi and P. caballi.

As a final proof of the difference between the two parasites, we decided to test if an animal which had recovered from the symptoms of "biliary fever" produced by the one would prove immune to infection with the other parasite.

A horse inoculated with *P. caballi* on 22. VI. 1910 suffered from a mild infection, and to all outward appearances made a good recovery. On 2. VIII. 1910[1] this horse was inoculated with *N. equi* with the result that it died of "biliary fever" induced by this parasite 20 days later. Only *N. equi* could be found in this horse's blood, although it might have been expected that *P. caballi* would have reappeared. This was the only one of our six horses which had haemoglobinuria, and it is possible that the mixed infection which existed was responsible for this symptom. It is desirable to determine if haemoglobinuria occurs in a pure *N. equi* infection; it probably does, since the animals may show jaundice, but there may be a difference in respect to the frequency and severity of this symptom.

It would be of considerable interest to determine if trypanblue affects *P. caballi* in a similar manner to *P. canis* and *P. bovis*[2] as one of us has shown elsewhere. The dye may, moreover, be found to influence *N. equi* differently to *P. caballi.* We have been unable to investigate this problem owing to lack of means with which to continue the research[3].

We have pleasure in acknowledging the kind assistance given to us

[1] Forty-two days after it had been inoculated with *P. caballi.*

[2] *Vide* Nuttall and Hadwen, *Parasitology*, II. 156–191, 229–235, 236–266 and Nuttall, *Ibid.* II. 409–434 and III. 202–209.

[3] *Note whilst going through the press:* Dr Yakimoff, February, 1912, in a letter to one of us, states that Bielitzer has recently treated equine piroplasmosis successfully in Russia by means of trypanblue, following the suggestion of Nuttall and Hadwen. This dye has now been found of value in the treatment of canine, bovine and equine piroplasmosis.

by Sir John McFadyean and Dr E. J. Marzinowsky, who sent us the strains of parasites, *N. equi* and *P. caballi*, respectively, with which we worked. H. B. Fantham, D.Sc., and Miss A. Porter, D.Sc., and Mr B. G. Clark aided considerably in the earlier part of the investigation, and our thanks are also due to them for their assistance. We are furthermore indebted to Mr Joseph Barcroft, F.R.S., for the kind help he gave us in determining the haemoglobin content of the blood of our horses; he has supplied us with the Note on the method we employed which will be found described at the end of the Appendix.

In a future paper we shall give a general summary of our present knowledge regarding Nuttalliosis and Piroplasmosis in horses as observed in different parts of the world.

Conclusions.

The term "Biliary Fever" or "Piroplasmosis," hitherto supposed to apply to a specific disease affecting horses, in reality refers to two distinct diseases produced by distinct parasites. For convenience' sake, and in accordance with the terminology at present in vogue, these two diseases may be named after the parasites which produce them, *i.e.* Piroplasmosis (due to *Piroplasma* [or *Babesia*] *caballi* Nuttall, 1910) and Nuttalliosis (due to *Nuttallia equi* (Laveran, 1910), França, 1909).

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Nuttall, G. H. F. and Strickland, C. (7. XII. 1910). Die Parasiten der Pferdepiroplasmose resp. der "Biliary Fever." Vorläufige Mitteilung. *Centralbl. f. Bakteriol. u. Parasitenk.*, Originale. LVI, 524–525.

DESCRIPTION OF PLATE III.

Nuttallia equi. Illustrating chiefly its mode of multiplication in the circulating blood, as shown in Giemsa-stained blood-films. Drawings in water-colour, made by C. Strickland with the aid of a camera-lucida and selected from a large number of figures made from freshly-stained films.

Fig. 1. Small free parasite.

Fig. 2. Small parasite shortly after its entry into a corpuscle.

Figs. 3–8. Successive stages in the growth of parasites which may be either amoeboid (Figs. 5, 6) or assume more or less temporarily the piriform shape (Figs. 4, 7, 8).

Figs. 9–19 indicate successive stages in nuclear division. The chromatin mass, which has hitherto been rounded or oval, becomes elongated and bent in conformity with the external contour of the parasite (Figs. 9, 10); the terminal and middle portion of the chromatin band becomes swollen (Fig. 11), and the strands connecting the three growing masses become thinner (Fig. 12), whilst the central mass in turn sub-divides. In some cases (Figs. 13–15) the strand leading to one of the smaller masses of chromatin ruptures before the central mass shows signs of division; in other cases (Figs. 16, 17) the central mass may show signs of division at this stage. In the next stage (Figs. 18, 19) that which was the central mass is clearly divided, but a fine chromatin thread still connects the daughter nuclei.

Figs. 20–22. Nuclear division is here complete, there being four distinct masses of chromatin which are usually of about the same size; the blue-staining protoplasm is beginning to collect around each nucleus.

Fig. 23. A typical cross-form, with the chromatin masses situated at the ends of the cross which is formed of four pear-shaped elements joined at their tapering extremities.

Figs. 24, 25. The four young parasites have now separated within the corpuscle, no residual body being discernible.

Figs. 26–28. Groups of four parasites derived from cross-forms which have escaped from ruptured corpuscles. In Fig. 28 one of the four parasites has become separated from the group.

Fig. 29. Two free parasites which would presumably have entered a fresh corpuscle either singly or together.

Figs. 30–32. Stages of division leading up to the formation of three or possibly four parasites.

Figs. 33, 34. Groups of three small parasites inside (33) and outside (34) a corpuscle.

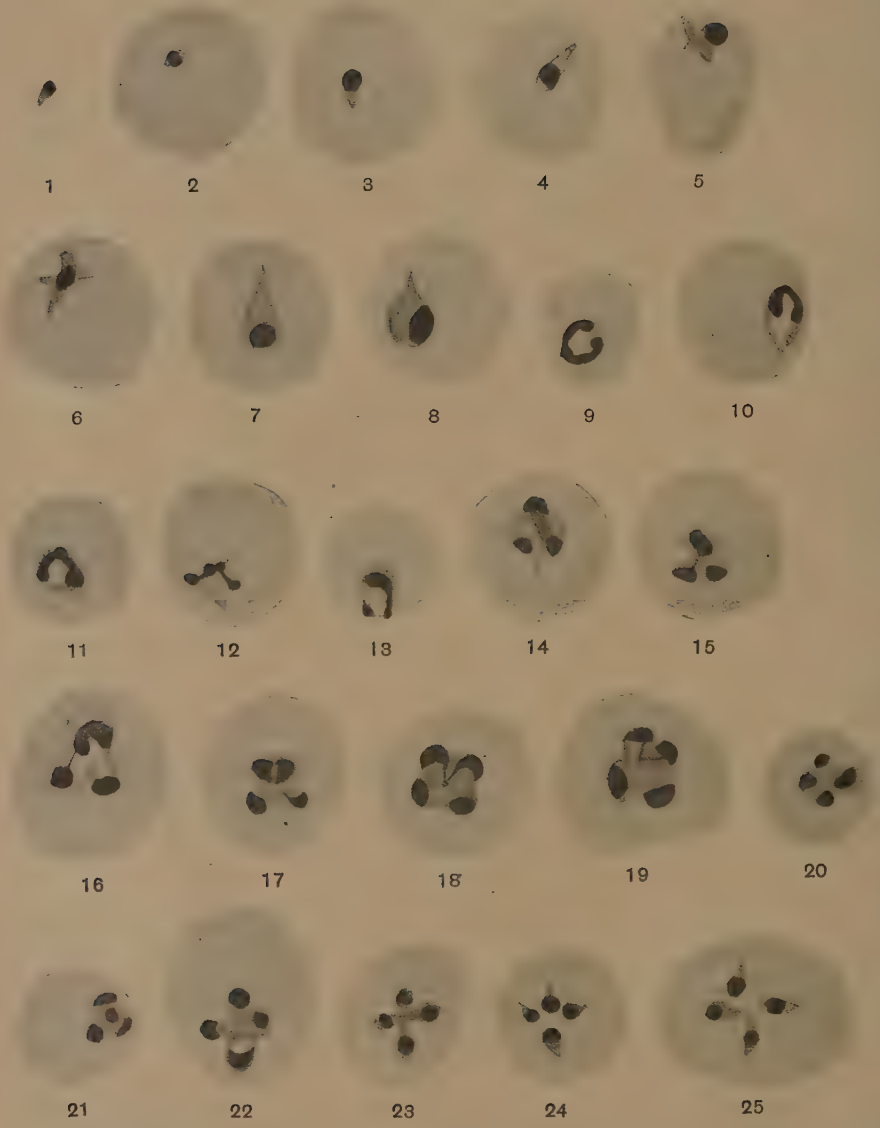
Fig. 35. Unusual appearance presented by two fairly large parasites, both of which are preparing to undergo division as evidenced by the structure of the chromatin. These parasites would have presumably given rise to six or eight parasites.

Figs. 36–40. Rounded, piriform or amoeboid parasites which show the chromatin drawn out and situated peripherally; 36 and 37 show a central vacuole.

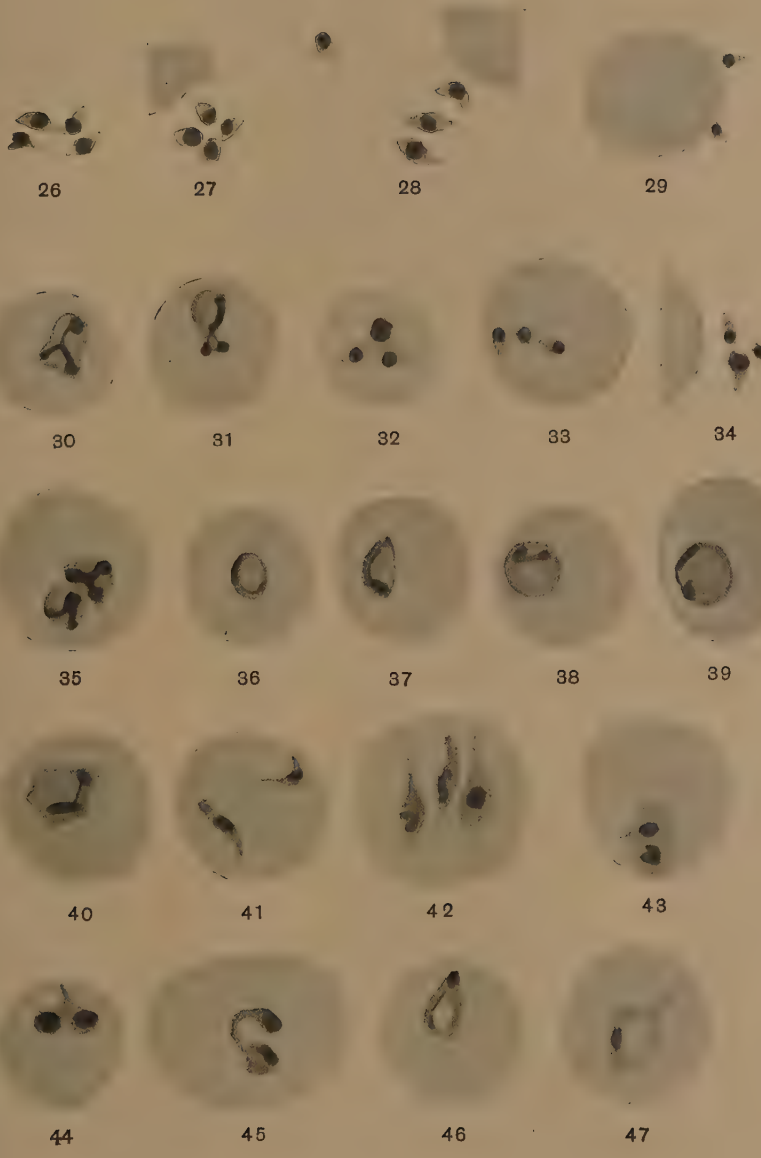
Figs. 41, 42. Corpuscles containing two and three parasites respectively.

Figs. 43–45. Appearances suggesting that simple division of one cell into two may occasionally take place.

Figs. 46, 47. Single piriform parasites closely resembling *Piroplasma.*



C. Strickland, del.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APPENDIX.

Containing the Protocols and Temperature Charts of *N. equi* Horses I—III and *P. caballi* Horses I—III.

Nuttallia equi Horse I.

The horse was inoculated subcutaneously with 5 c.c. of citrated blood at 10 a.m. on 27. xi. 1909; the horse died 7. xii. 1909 (day 11).

Day	Temp. °F. M.	Temp. °F. E.								
1	99·2	—	Inoculated.							
2	100	—								
3	99·6	—								
4	99·6	—								
5	99·4	—								
6	100	—								
7	101·2	—								
8	102·6		Parasites appeared, very few, only 5 infected r.b.c. along 2 film-edges.							
			% r.b.c. infected	% (S)	% (M)	% (L)	% (D)	% (2—4)	% (F)	
8		103·4	0·57	29	34	32	0·4	2	•	
9	102·4		0·72							
		103·8	1·4	8	32	47	•	5	8	
10	105		4·8	2	50	36	2	3	1	
		105·8	5·4	3	31	62	•	4	4	8.30 p.m. horse weak on hind legs.
11	105		3·7	29	39	17	•	5	10	2 p.m. ditto, not feeding.
		104	3·9	55	11	4	0·4	3	26	6 p.m. prostrate; too weak to stand. Killed.

The signs denote corpuscles containing

(S)	=	A small intracorpuscular parasite	rounded, piriform or amoeboid.
(M)	=	A medium-sized ditto	
(L)	=	A large sized ,,	
(D)	=	Dividing forms and cross-forms.	
(2–4)	=	Two to four intracorpuscular parasites.	
(F)	=	Free parasites.	

From the following chart it will be seen that the parasites were first detected in the horse's blood on the 8th day after inoculation; their number rose so that about 5 % of the corpuscles were infected on the 10th day, whilst on the day following about 4 % were infected. With regard to the percentage of different types of parasites encountered, one striking fact emerges, namely, that, relatively speaking, the number of free and small parasites greatly increased toward death and the number of medium and large sized parasites decreased. The rise and fall in the percentage of infected corpuscles accompanied the rise and fall in the body temperature.

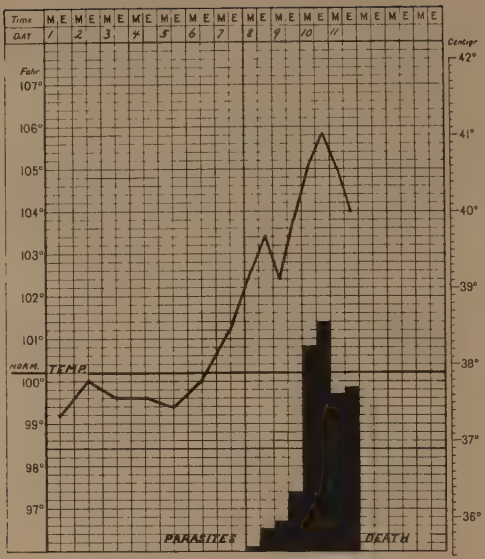

Chart of *Nuttallia equi* Horse I.

N. equi Horse II.

Horse inoculated subcutaneously with 5 c.c. of defibrinated jugular blood from Horse I at 5 p.m. 8. XII.1909. (Blood kept on ice 12 hours.)

Day	Temp. °F. M.	Temp. °F. E.	
1	—	—	Inoculated.
2	96·6	—	
3	100·6	—	
4	99·8	—	
5	99·6	—	
6	99·4	—	
7	102·6	102·6	Parasites appear, 0·04 % r.b.c. infected.
8	102·6	101·4	,, Morning 3 (O); Evening 5 (O).
9	101·0	101·8	,, ,, 19 (O), 1 (+), 1 (P).
10	104·2	103·8	,, ,, 0·9 % r.b.c. infected; Evening 0·7 %.
11	105·2	105·4	,, ,, 0·8 % ,, ,,
12	103·4	103·4	,, only 1 (O).
13	100·8	100·8	,, ,, 3 (O).
14	99·2	99·3	,, none found.
15	99·4	99·6	,, only 4 (O).
16	99·6	99·8	,, ,, 3 (O).
17	99·2	—	,, 14 (O), 1 (P).
18	98·8	—	,, 6 (O).
19	98·8	—	,, none found.
20	98·6	—	,, ,, ,,
21	99·4	—	,, 20 (O), 2 (P).
22–57	Normal		,, none found on 28 out of 35 days, temperature not taken on 7 days out of the 35.
58–148	,,		Not examined for parasites; temperature recorded on 61 out of the 90 days.

The signs (O), (P) and (+) indicate, respectively, single round, piriform (or oval) and cross-forms contained in corpuscles.

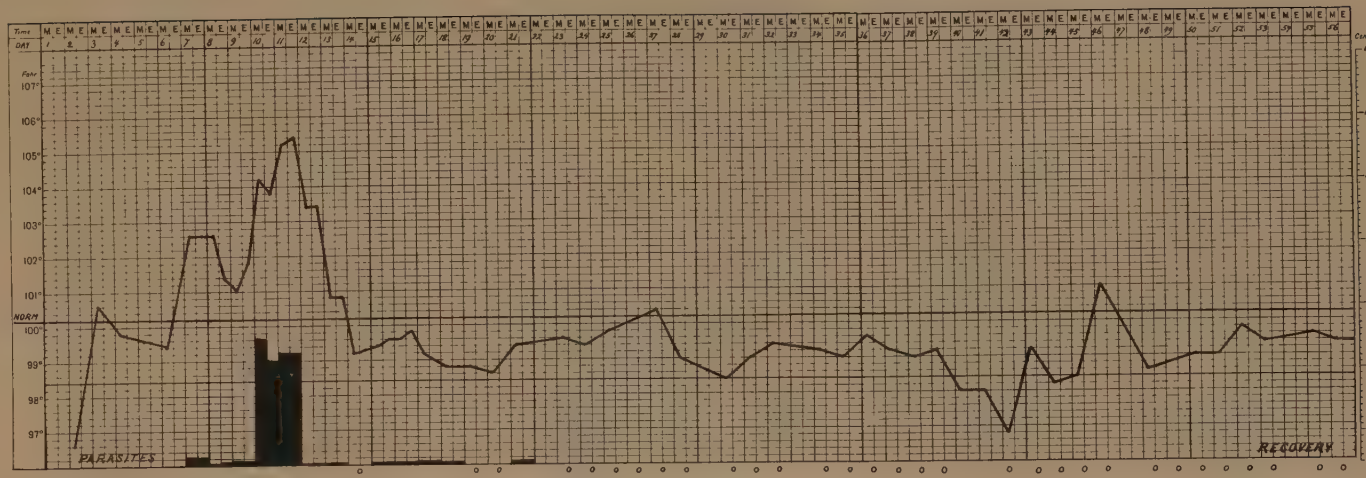

Chart of *Nuttallia equi* Horse II.

Reference to the foregoing chart shows that the parasites appeared with the onset of fever on the 7th day following the inoculation. The highest temperatures and highest percentages of infected corpuscles (only about 1 %) were both attained on the 10th and 11th days, after which the temperature fell gradually to normal and the parasites dwindled in numbers. The small number of parasites precluded any enumeration of the relative percentages of the different types of parasites present in the blood. This animal made a complete recovery, and is alive and well to-day (27 February, 1912).

Nuttallia equi Horse III.

The Horse was inoculated subcutaneously with 20 c.c. of defibrinated blood taken from the jugular vein of Horse II at mid-day on 2. VIII. 1910. Horse III had previously been infected with *Piroplasma caballi* on 22. VI. 1910, but had recovered (see Protocol of *P. caballi* Horse II). *N. equi* appeared in the horse's blood on the 10th day, and it died at 5.30 p.m. on the 20th day after inoculation (21. VIII. 1910).

Day	Temp. °F. M.	Temp. °F. E.	% r.b.c. infected	% (S)	% (L)	% (D)	% (2—4)	% F		R.b.c. per c.mm.	Haemoglobin	Leucocytes per c.mm.*
1	97·8	—							Inoculated.			
2	98·2	—										
3	98·2	—										
4	97·2	—										
5	98·2	—										
6	99·0	—										
7	98·2	—							.....	8,888,000	0·227	8,640
8	98·2	—							.....	8,872,000	0·230	12,900
9	97·4	—							.....	8,968,000	0·222	7,120
10	100·4	—							Parasites appeared.....	9,155,000	0·240	7,800
10		102·0	0·2	34	65	1	·	·				
11	103·0		3·0	23	75	1	·	0·4		9,004,000	—	10,650
		104·2	2·0	43	50	4	2	·	Horse weak.			
12	104·2		—	—	—	—	—	—	Horse weak, jaundice; off	7,240,000	0·230	9,540
		106·6	11·6	23	70	3	4	1	his food.			
13	105·0		10·3	47	45	0·6	2	5	Very weak; haemoglobin-	5,390,000	—	—
		104·6	6·8	50	43	2	3	2	uria; blood appears watery, coagulation retarded.			
14	103·0		8·4	56	38	0·2	4	1	Rather better; feeding better,	5,365,000	0·141	12,000
		101·7	10·6	62	33	2	2	1	haemoglobinuria.			
15	100·6		5·2	31	65	1	3	0·4	Ditto; urine smoky.	4,812,000	0·104	7,520
		102·4	7·4	28	66	0·6	5	0·6				
16	102·4		9·2	15	80	0·6	3	0·2	Very weak, especially hind	3,840,000	0·115	9,700
		100·8	6·8	35	57	1	4	1	legs, which are stretched apart.			
17	103·0		8·0	19	75	1	3	0·2	Seems better, feeding; swell-	3,848,000	0·104	13,500
		103·0	12·8	28	67	2	3	·	ing on jaw (abscess).			
18	103·0		8·6	25	69	1	4	0·3	Weak; jaundice persists.	3,230,000	0·100	22,600
		102·2	13·2	20	73	2	5	·				
19	103·2		6·6	23	74	1	2	·	Very weak, lying down.	3,120,000	0·066	33,600
		101·0	4·8	20	75	1	4	·			Mean of two readings, being the O capacity of 1 c.c. of blood in c.c.	
20	98·4		8·4	29	53	2	11	6	Helpless, urine smoky; found dead at 5.30 p.m.			

* Average number of counts = 5.

The signs		denote corpuscles containing
(S)	=	small and medium sized piriform or oval parasites.
(L)	=	large rounded parasites.
(D)	=	dividing and cross-forms.
(2–4)	=	two or more parasites.
F	=	free forms.

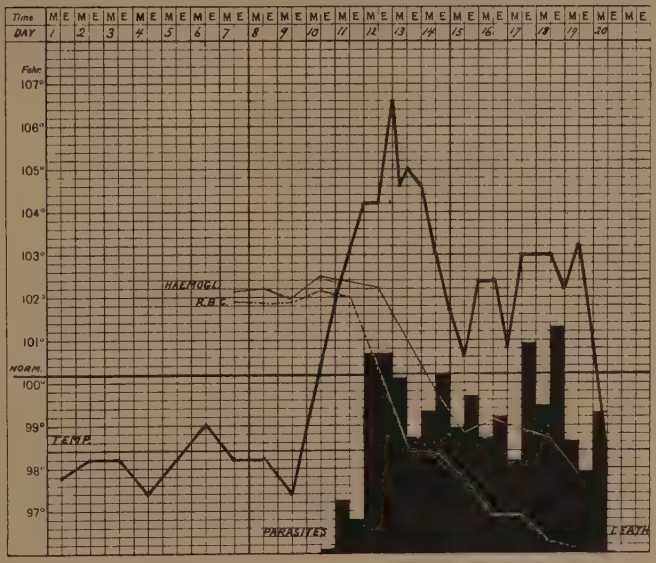

Chart of *Nuttallia equi* Horse III.

Changes in the blood.

Day	Differential leucocyte count, %					Nucleated r.b.c.
	Lymphocytes	Large mono's	Transit.	Polym's	Eosin's	
8	27	32	2	26	13	0
14	20	29	8	43	0	0
17	26·5	34	14	22	1	3
19	22	34	7·3	34·4	0	3·3

Piroplasma caballi Horse I.

The Horse was inoculated subcutaneously at 3 p.m., 4. VI. 1910, with 15 c.c. of citrated blood sent to us by Dr E. J. Marzinowsky, from Moscow. The horse died on the 22. VI. 1910 (day 19).

Day	Temp.°F.	
1	—	Inoculated.
2	100·4	
3	97·8	Abscess forming at seat of inoculation.
4	99·1	
5	97·4	Abscess lanced.
6	96·8	
7	97·4	
8	96·2	
9	97·0	
10	97·2	
11	96·8	
12	97·4	
13	97·8	
14	99·8	
15	101·0	Parasites appeared, only 2 (PP) found after long search.
16	99·6	
17	100·0	Parasites present, only 3 (PP) found.
18	101·4 (a.m.)	,, ,, few, 66 % (O), 34 % (PP).
	102·4 (p.m.)	
19	—	Horse found dead but still warm at 9 a.m. *Died of pulmonary haemorrhage.*

There were too few parasites present in this horse's blood to render a satisfactory blood-count possible during life. Of the parasites found on the 18th day, 66 % were single round parasites (O) and 34 % double piriforms (PP). It was possible, however, in stained films prepared from the internal organs after death, to make an enumeration of the different types of parasites as follows :

	(O)	(D)	(PP)	Free
Liver	84	1	8	7
Spleen	73	2	10	15
Lung	77	0	3	20

Piroplasma caballi Horse II.

The horse was inoculated subcutaneously at 5.30 p.m., 22. vi. 1910, with 20 c.c. of defibrinated blood taken from the jugular vein of *P. caballi* Horse I very soon after death. Recovered quickly.

Day	Temp. °F. a.m.	p.m.	
1	97·2	—	Inoculated.
2	96·8	—	
3	99·2	—	
4	98·2	—	
5	97·2	—	
6	97·6	—	
7	100·8	—	
8	102·4	103·4	Parasites found, only 1 (O), 3 (PP). Horse off his feed.
9	100·8	101·8	No parasites found.
10	96·8	97·4	,, ,,
11	100·4	102·4	,, ,,
12	100·8	101·4	,, ,,
13	98·8	98·6	,, ,,
14	100·2	98·4	,, ,,
15	100·2	100·0	,, ,,
16	99·8	99·6	,, ,,
17	99·8	99·8	,, ,,
18	97·6	100·6	,, ,,
19	Normal		,, ,, The horse recovered and showed no subsequent rise of temperature. Forty-two days after it was inoculated with *P. caballi* blood it was inoculated with *N. equi* blood; the further history of the horse is given under *N. equi* Horse III.

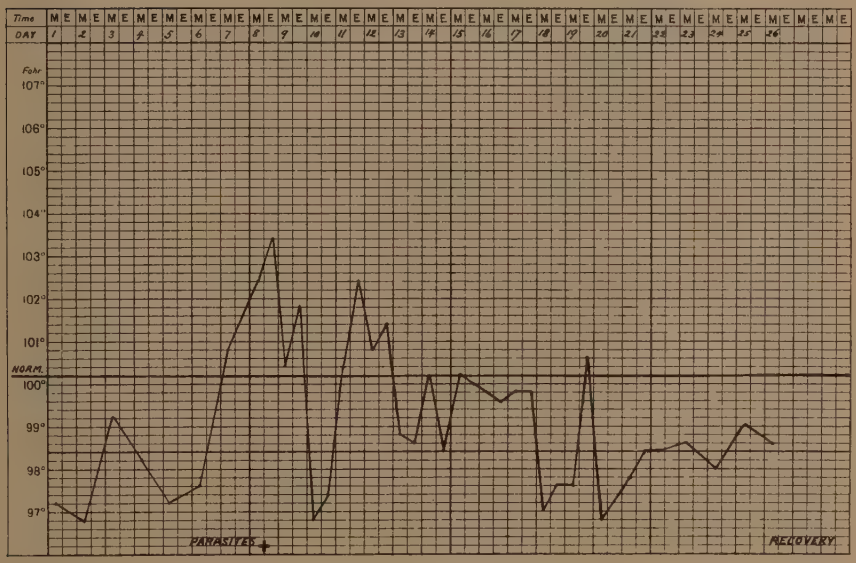

Chart of *Piroplasma caballi* Horse II.

In this horse the temperature began to rise on the 7th day and parasites were found on the 8th day, although only four infected corpuscles were encountered in a blood-film. No parasites were detected after the 10th day, and the temperature soon returned to normal.

Piroplasma caballi Horse III.

The Horse was inoculated subcutaneously at 12.30 p.m., 18. VII. 1910, with 20 c.c. of defibrinated blood taken from *P. caballi* Horse II. The horse died at 2.30 p.m., on 5. VIII. 1910 (day 19).

Day	Temp. °F. a.m.	Temp. °F. p.m.	No. of infected r.b.c. per 30,000	% (P)	% (O)	% (D)	% (PP)	% F	
1	99·8	—							Inoculated.
2	98·8	—							
3	99·2	—							
4	100·2	—							
5	99·0	—							
6	99·0	—							
7	99·0	—							
8	98·6	—							
9	100·8								
		103·0							
10	103·4								Parasites appeared, only 1 (PP) found in film.
		103·2							,, ,, ,, 1 (PP) ,, ,,
11	102·2								,, ,, ,, 1 (P), 1 (PP) found in film. Feeding less.
		101·8							
12	102·2								,, ,, ,, 1 (O), 1 (PP) ,, ,,
		102·0							
13	100·0	—	6						
14	99·0	—	3						Horse feeding very little.
15	104·6	—	12	(P)	(O)	(D)	(PP)	F	,, ,, ,,
16	105·4	—	6	6	71	2	21	0	,, ,, ,,
		105·6	12	6	47	5	42	0	
17	105·4		4	13	67	4	15	1	Horse weak on hind legs, drinks
		105·0	60	5	64	4	27	0	much.
18	103·6		85	10	68	4	18	0	Horse cannot stand; icterus;
		102·2	80	4	57	7	31	0	spleen large and tender.
19	102·4		150 = 0·5 %	2	51	7	39	1	Ditto, some normal urine passed.
		—							Treated at 1.30 p.m. by subcutaneous injection of 150 c.c. of 1·5 % Trypanblue solution. Horse died at 2 p.m.

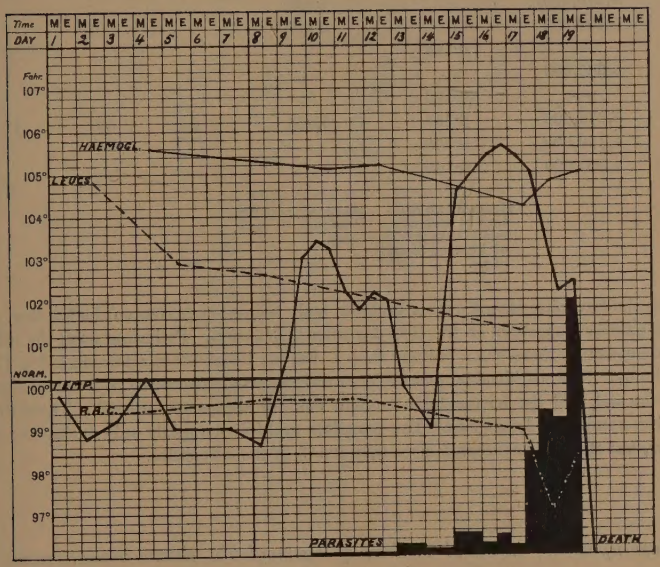

Chart of *Piroplasma caballi* Horse III.

Changes in the blood.

Day	R.b.c. per c.mm.	Haemoglobin	Leucocytes per c.mm.	Differential count of leucocytes		
				Eosin's	Neutroph's	Mono's and lympho's
1	—	—	—	30	53	17
2	—	—	8,800			
3	6,400,000	—	—			
4	—	0·207	—			
5	6,570,000	—	6,850			
8	6,700,000	—	6,600			
10	—	0·189	—			
11	6,685,000	—	—			
12	—	0·192	—			
17	5,872,000	0·155	5,300	0	83	17
18	4,140,000	0·178	—			
19	4,960,000	0·185	—			
		Mean of two readings in each case, being O capacity of 1 c.c. of blood in c.c.	Mean of ca., ten readings in each case.			

From the foregoing record we see that the body temperature rose on the 9th day, and that the parasites appeared on the 10th day after inoculation. Following upon the initial rise of temperature there was a fall and then a rise, the temperature attaining 105·6° F. on the 16th day. The red blood corpuscles were not materially decreased in numbers except toward the end, and the haemoglobin content was not appreciably altered. There was a fall in the number of leucocytes. The parasites multiplied rapidly only during the last 36 hours, but they never infected more than 0·5 % of the corpuscles.

Note upon the chemical method of haemoglobin determination used in this investigation.

The oxygen capacity of the blood was investigated by direct measurement of the oxygen which it contained when shaken with air. The method employed was that form of the differential method described by Barcroft and Roberts (*Journ. of Physiology*, xxxix. p. 435, 1910) and based upon the observation of Haldane, that ferricyanide of potassium liberated, from an alkaline solution of haemoglobin, an amount of oxygen equal to the respiratory oxygen contained combined with the pigment.

The method is briefly as follows:

Into each of the bottles is placed 0·2 c.c. of dilute ammonia solution (made by adding 1 c.c. of NH_3, SG ·880 to 250 c.c. of water) and about 0·1 c.c. of blood, accurately measured from a pipette. The present experiments were performed with 0·082 c.c. The fluids are gently shaken so that the blood becomes laked and thoroughly oxidised. The bottles may then be placed on the apparatus, but before this is done a drop of potassium ferricyanide is placed in the pouch projecting from the side of one of the bottles. The

ferricyanide is introduced with a curved pipette, and care must be taken to see that no mixture takes place between the solution of ferricyanide and haemoglobin. The apparatus is now hung on the edge of a vessel full of water so that the bottles are completely immersed. After ten minutes the taps, which hitherto have allowed both the manometer and the bottles to be in communication with the external air, are closed so that the two former are in communication with one another but not with the air outside. The fluid surfaces in the manometer are read to ascertain if any zero error exists. They are now at the same level. The ferricyanide and the haemoglobin are mixed and the apparatus shaken for a minute and then replaced in the bath. Probably three such shakings suffice to liberate all the oxygen. The observed difference of pressure is noted. The estimation is then repeated with the blood in the other bottle.

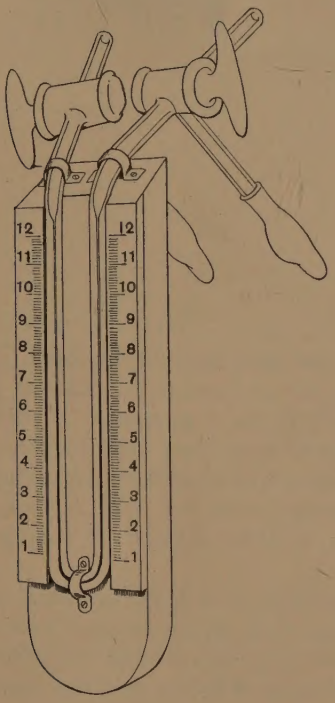

Fig. 1. Showing the apparatus used in the method here described.

To the right of the figure is seen one and part of another of the small flasks in which the mixture of blood, ammonia, and ferricyanide is made. The manometer and the flasks and the air are seen to be connected by three-way passages governed by three-way cocks. The dark line in the manometer represents the oil. (Figure reproduced from the *Journ. of Physiol.*, by kind permission of Professor Langley.)

It is necessary to know how much oxygen corresponds to a difference of pressure of 1 mm. on the scale. For this purpose a known volume of oxygen is liberated in the apparatus (about 0·2 c.c.). An experiment similar to that described, but with different fluids, is performed. The blood is replaced by hydrogen peroxide, the ammonia by water and the ferricyanide by potassium permanganate (approximately 1 c.c. = 0·003 g. permanganate). The hydrogen peroxide must first be carefully standardised by titration with standard potassium permanganate in the presence of sulphuric acid with which reagent it gives off its oxygen as follows :

$$K_2Mn_2O_8 + 5H_2O_2 - 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 5H_2O + 5O_2.$$

In the apparatus itself it is better to dispense with the sulphuric acid. The relation of 316 grams of permanganate to 1110·00 c.c. of oxygen remains true.